The role of interleukin-36 in skin and systemic inflammation

Catapano, Marika

Awarding institution:
King's College London

The role of interleukin-36 in skin and systemic inflammation

Marika Catapano

Genetics and Molecular Medicine PhD

King's College London

May 2019

Table of Contents

Table of Contents 1

Tables 4

Figures 5

Contributions 7

Abbreviations 8

1 Abstract 12

2 Introduction 14

2.1 Autoimmunity and autoinflammation 14
2.1.1 The immune system 14
2.2 IL-36 cytokines 29
2.2.1 Overview 29
2.2.2 Expression and processing of IL-36 29
2.2.3 IL-36R signal transduction 32
2.2.4 The immune function of IL-36 35
2.3 Psoriasis 40
2.3.1 Plaque Psoriasis 43
2.3.2 Generalised Pustular Psoriasis 50
2.4 Aims 58
3 Materials and Methods 59

3.1 Materials 59
3.2 Study resources 60
3.3 Transcription profiling 65
3.3.1 Retrieval of publicly available datasets 65
3.3.2 RNA isolation and sequencing 67
3.3.3 Differential expression analysis 68
3.3.4 Enrichment analyses 70
3.4 Cell culture and stimulation 71

3.4.1 Cell isolation 71
3.4.1 Cell culture 72
3.4.2 Quantitative real-time PCR 72
3.4.3 ELISA 74
3.5 Flow cytometry 75
4 IL-36 cytokines have a key role in the pathogenesis of psoriasis 80
4.1 IL-36α, -β and -γ induce similar signature genes in keratinocytes 80
4.2 IL-36 is a dominant cytokine in the GPP and Ps skin transcriptomes 89
4.3 IL-36 induced genes are enriched within Ps susceptibility loci 92
4.4 IL-36 signature genes cluster to pathways implicated in the pathogenesis of psoriasis 94
4.5 Validation of selected IL-36 target genes 99
4.6 Discussion 101
5 The Role of IL-36 in systemic inflammation, a transcriptomic approach 104
5.1 The whole blood transcription profiles of GPP patients are very heterogeneous 104
5.2 IL-36 signalling is systemically up-regulated in GPP patients, regardless of mutation status 107
5.3 Type-I-IFN related pathways are abnormally active in GPP 109
5.3.1 Genes related to IFN signalling are enriched in the GPP transcriptome 109
5.3.2 Type-I IFN regulatory networks are active in the GPP whole-blood transcriptome 112
5.3.3 INF-α related transcription factors drive gene up-regulation in GPP 114
5.4 Overlap between the genes that are up-regulated in GPP and interferonopathies 116
5.5 The IFN score is elevated in psoriatic cases compared to controls 118
5.6 IL-36 and IFN scores are highly correlated 123
5.7 Discussion 125
6 The Role of IL-36 in systemic inflammation, experimental validation 127
6.1 The IL-36 receptor is robustly expressed on the surface of plasmacytoid dendritic cells 127
6.2 IL-36 potentiates IFN-α production 131
6.3 IL-36 up-regulates PLSCR1 expression in pDCs 134
6.4 Discussion 136
7 Discussion 140
7.1 Transcriptomic to unravel mechanisms of inflammatory diseases 140
7.2 The pathogenic role of IL-36 142
7.3 IL-36 as a therapeutic target in psoriasis 144

8 References 145

9 Appendix 159

Tables

Table 3.2. 1. Demographics and clinical characteristics of the affected individuals 59
Table 3.2. 2. Demographics of the healthy individuals used as controls..... 64

Table 3.3. 1. Study resources 66

Table 3.4. 1. Primers used for the real time PCR 73

Table 3.5. 1. Flow Cytometry Antibodies 76

Table 4.1. 1. RNAseq coverage statistics 81

Table 5.1. 1. Whole-blood RNAseq statistics. 105

Figures

Fig. 2.1.1. Innate and adaptive immunity. 15
Fig. 2.1.1.1 Toll-like receptors family 19
Fig. 2.1.1.2. The immune-mediated disease continuum, with examples. 21
Fig. 2.1.1.3. T cells phenotypes. 25
Fig. 2.1.1.3.1. B cell development. 27

Fig. 2.2.1. Overview of IL-36 signalling. 31
Fig. 2.2.2. IL-36R activation and downstream signalling cascade. 33
Fig. 2.2.3. Effect of IL-36 on different tissues and tissue-specific disorders. 39

Fig. 2.3.1. Classification of psoriasis. 42
Fig. 2.3.2. Current understanding of plaque psoriasis pathogenesis 46
Fig. 2.3.3. Psoriasis targeted immunotherapy. 49

Fig. 2.3.2.1. Histology of pustular psoriasis. 52
Fig. 2.3.2.2. IL-36 signal transduction. 55

Fig. 3.5.1. Gating Strategy. 78

Fig. 4.1.1. Features of IL-36α, -β and -γ transcriptomes in keratinocytes. 83
Fig. 4.1.2. Differentially expressed genes in keratinocytes treated with IL-36 cytokines. 85
Fig. 4.1.3. Identification of IL36_182 gene set. 87

Fig. 4.2.1.The IL36_182 set significantly overlaps with genes up-regulated in GPP or Ps. 90
Fig. 4.2.2. IL-4 signature genes do not overlap with the Ps or GPP transcriptome 91

Fig. 4.3.1. Members of the IL36_182 set are over-represented within psoriasis susceptibility intervals. 93
Fig. 4.4.1. There is a substantial overlap between the pathways that are enriched in the IL-36α, -β and -γ transcriptomes. 95
Fig. 4.4.2. The biological pathways driven by IL-36α, -β and -γ are relevant to the pathogenesis of psoriasis. 96
Fig. 4.4.3. IL-36 induced genes map to pathways related to psoriasis. 98

Fig. 4.5.1. Validation of up-regulated genes in keratinocytes obtained from independent healthy donors. 100

Fig. 4.6. 1.IL-36 enhances skin inflammation by sustaining IL-17 production. 103

Fig. 5.1.1. Principal component analysis (PCA) of whole-blood RNA-seq data 106

Fig. 5.2.1. The IL-36 score is significantly increased in psoriatic patients compared to controls. 108

Fig. 5.3.1. Genes that are differentially expressed in the whole blood of GPP patients. 110

Fig. 5.3.2. Interferon signalling is the most significantly enriched pathway in GPP whole-blood..... 111
Fig. 5.3.3. Transcriptional modules related to innate responses are enriched among genes over-expressed in GPP..... 113
Fig. 5.3.4. Key Type-I IFN regulators driving gene up-regulation in GPP. 115

Fig. 5.4.1. Overlap between genes that are over-expressed in GPP and other autoinflammatory diseases. 117

Fig. 5.5.1. The IFN score is elevated in psoriatic patients compared to controls. 119
Fig. 5.5.2. The analysis of an extended dataset validates the abnormal IS observed by RNA-seq..... 121
Fig. 5.5.3. Expression of interferon signature genes across individuals of different descent. 122

Fig. 5.6.1. IL-36 and IFN scores show significant positive correlation. 124

Fig. 6.1.1. IL-36R expression in adaptive immune cells. 128
Fig. 6.1. 2. IL-36R expression in innate immune cells. 129
Fig. 6.1.3. IL-36R expression summary data. 130

Fig. 6.2. 1. IL-36 pre-treatment enhances IFN-α responses in PBMCs..... 132
Fig. 6.2. 2. IL-36 potentiates IFN-α production in pDCs but not B cells. 133

Fig. 6.3.1. IL-36 up-regulates the expression of PLSCR1. 135

Fig. 6.4.1. IL-36/IFN-α axis: proposed model. 139

Contributions

I carried out all the work presented in this thesis, with the following exceptions. Dr Satveer Mahil stimulated primary keratinocytes with IL-36, prior to the RNA-sequencing experiment described in chapter 4. She also validated the expression of IL-36 target genes by means of real-time PCR (section 4.5). Mrs Marta Vergnano contributed to the PBMC and pDC stimulations described in chapter 6. It has been a pleasure to work with these colleagues and I am immensely grateful for their contributions to this thesis.

Abbreviations

AGEP	Acute Generalised Exanthematous Pustulosis
AGS	Aicardi-Goutiéres Syndrome
APC	Antigen Presenting Cell
APS	Autoimmune Polyendocrine Syndrome
BCG	Mycobacterium bovis Bacillus Calmette-Guérin
CANDLE	Chronic Atypical Neutrophilic Dermatosis with Lipodystrophy and Elevated temperature
DC	Dendritic Cell
DEG	Differentially Expressed Gene
DIRA	Deficiency of Interleukin 1 Receptor Antagonist
DITRA	Deficiency of Interleukin Thirty-six-Receptor Antagonist
DSS	Dextran-Sulfate Sodium
FC	Fold Change
FDR	False Discovery Rate
GEO	Gene Expression Omnibus
GPP	Generalised Pustular Psoriasis
GWAS	Genome Wide Association Studies
HLA	Human Leukocyte Antigen
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IPA	Ingenuity Pathway Analisys
IPEX	Immunodysregulation Polyendocrinopathy Enteropathy X-linked
IS	Interferon Score
ISG	Interferon Signalling Gene
KC	Keratinocyte
LPS	Lipopolysaccharide
LRR	Leucine-Rich-Repeats
MAPK	Mitogen-Activated Protein Kinase
mDC	myeloid Dendritic Cell
MDDC	Monocyte-Derived Dendritic Cell
MFI	Mean Fluorescence Intensity
MHC	Major Histocompatibility Complex
NET	Neutrophil Extracellular Traps
NK	Natural Killer
NLR	Nod Like Receptor
NOMID	Neonatal Onset Multisystem Inflammatory Disease
P	P value
PBMC	Peripheral Blood Mononuclear Cell
PCA	Principal Component Analysis

PCR	Polymerase Chain Reaction
pDC	Plasmacytoid Dendritic Cell
PLUM	Pustular psoriasis: eLucidating Underlying Mechanisms
PPP	Palmoplantar pustulosis
PRR	Pattern Recognition Receptor
Ps	Plaque Psoriasis
PsV	Psoriasis Vulgaris
RLR	RIG-I like receptors
RPKM	Reads Per Kilobase per Million
SLE	Systemic Lupus Erythematosus
TCR	T Cell Receptor
Th	T helper
TIR	Toll IL-1 Receptor
TLR	Toll-like Receptor
TRAPS	TNF receptor-associated periodic syndrome
Type-I-IFNpathy	Type-I Interferonopathy

Acknowledgments

I would like to acknowledge the Psoriasis Association for giving me the opportunity to pursue this PhD and for their funding support.

I am incredibly grateful to my first supervisor, Dr Francesca Capon, for her wonderful supervision, wise guidance and inspirational mentorship. I am extremely lucky to have benefited from her support, patience and enthusiasm; she helped me mature as a scientist, and improve my communication and writing skills.

I was also privileged to be supervised by Prof. Francesca Ciccarelli, who gave me the opportunity to join her lab and taught me to always have a critical and analytical approach to science. Both my supervisors have been invaluable in critiquing this project and ensuring continual progress.

I would also like to extend my gratitude to Prof. Jonathan Barker and Prof. Catherine Smith for providing me with generous guidance.

I have been most fortunate to have developed collaborations with other brilliant researchers over the course of this PhD, who shared their time and knowledge to help progress this project. I would like to thank Dr Marco Romano and Prof. Giovanna Lombardi, who helped set FACS experiments and facilitated the generation of beautiful results. I would also like to thank Dr Thomas Tull and Prof. Jo Spencer together with Dr Paola Di Meglio for sharing their wisdom and ideas.

I would like to express further thanks to many other colleagues from King's College London, in particular to Thanos, Matteo and Lorena with whom I shared the first years of my PhD and have been fundamental during the start of this journey.

Thanks to Marta, Satveer and Sophie who introduced me to the “wet-lab world”, helped me with experiments and supported me during the most critical steps of the PhD. I am also glad to have met Natashia, Thanos, Karoliina, Charlotte and Dan. Even if our paths overlapped for a short time their contagious smiles and great advice have been important to me. It has been a privilege to be part of 9th floor and meet true friends and wonderful people. Thank you all for making this PhD such an extraordinary experience.

Finally, I would like to thank my wonderful family for their encouragement. Despite being far away, they supported me by sending me their affection and love. I am grateful to Amanda, Silvia, Elisa, Claudia, LeeAnn, Marta, Luigi, Angela and my sisters Lisa and Mena for believing in me in moments of uncertainty and for always being pleasant company. One additional essential person was Harry, who patiently assisted me with his positive energy, kindness and sagacity.

1 Abstract

Interleukin 36α, -β and –γ (hence IL-36) are a group of innate cytokines that play a key role in epithelial immune homeostasis. In fact, mutations causing excessive IL-36 signalling have been associated with generalised pustular psoriasis (GPP), a severe autoinflammatory disease presenting with flares of skin pustulation and systemic upset.

Given that GPP patients often suffer from concurrent plaque psoriasis (Ps), the hypothesis underlying this study was that abnormal IL-36 activity may also contribute to the pathogenesis of Ps. As the disease presents with cutaneous (red, scaly plaques) and systemic (increased cardiovascular risk) manifestations, the effects of IL-36 were examined in both contexts.

In the first part of the research, a signature of IL-36 activation was defined through the transcription profiling of primary keratinocytes treated with IL-36α, -β or γ. This identified a core set of transcripts that are up-regulated by all three cytokines. Importantly, these differentially expressed genes (DEG) showed an enrichment for pathways related to IL-17 signalling and leukocyte chemotaxis, two processes that are critical to the pathogenesis of Ps. There was also a marked overlap between the genes that are up-regulated by IL-36 and those that are over-expressed in Ps skin.

The second part of the study focused on the systemic effects of IL-36. These were initially investigated by whole-blood RNA-sequencing of GPP cases and unaffected controls. While the experiment confirmed the up-regulation of IL-36 dependent transcripts in GPP, it also showed an unexpected over-expression of Type-I IFN stimulated genes. Signatures of abnormal IL-36 and Type-I IFN activity were also observed in Ps leukocytes. Follow-up experiments demonstrated that IL-36 acts directly on plasmacytoid dendritic cells, where it up-regulates Toll-like receptor-9 signalling and IFN-α production.

Taken together, these findings demonstrate that IL-36 plays an important role in the cutaneous and systemic pathogenesis of Ps.

2 Introduction

2.1 Autoimmunity and autoinflammation

2.1.1 The immune system

Antimicrobial responses appear very early in evolution. Even simple organisms like prokaryotes have a defence system against infectious agents. However, vertebrates have evolved a highly adaptable immune system that confers protection against pathogens through an array of specialised molecules and cells. These mediate two interconnected responses known as innate and adaptive immunity [1]. Innate immunity activates an early and non-specific response, which is mediated by evolutionary conserved receptors, encoded in the germline. Conversely, adaptive immunity drives a delayed and highly-specific response, whereby antigen presenting cells enable T and B lymphocytes to specifically recognise the infectious pathogen (Fig. 2.1.1).

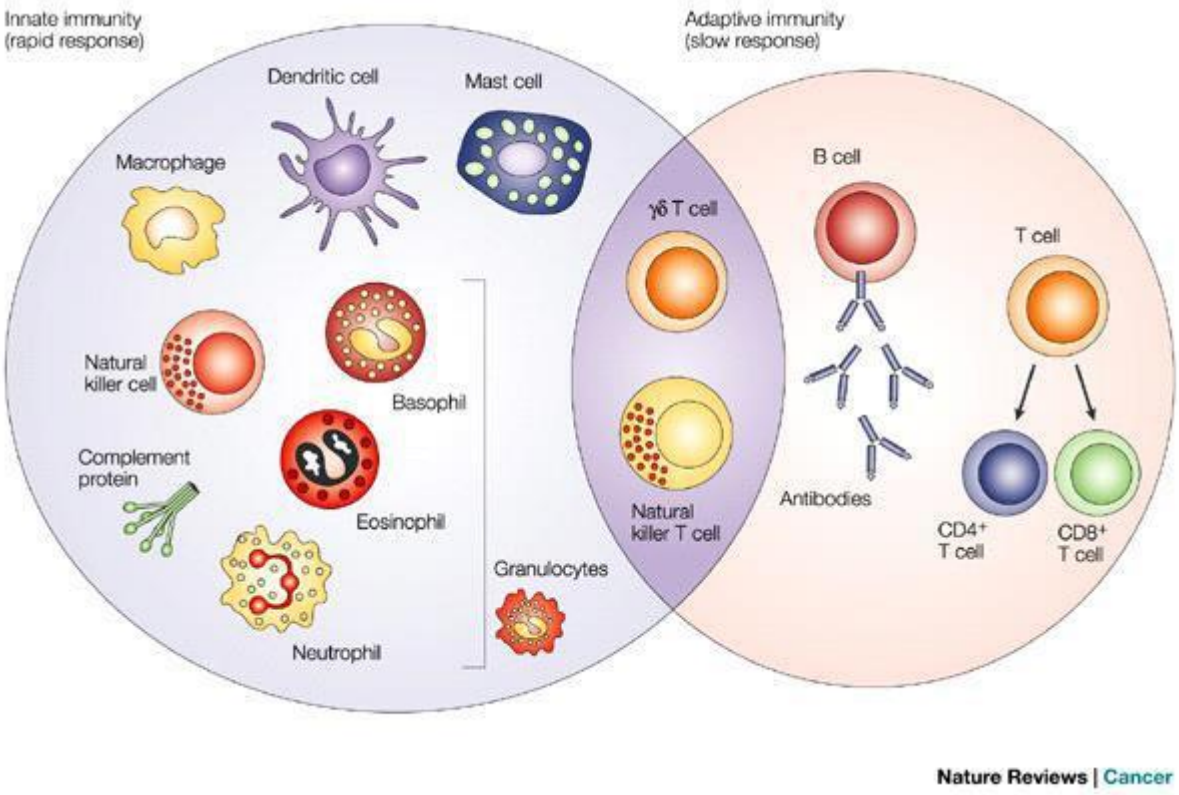


Fig. 2.1.1. Innate and adaptive immunity.

The figure (Dranoff et al. [2]) illustrates the different cell types mediating innate and adaptive immunity. While some cells can have a specialist function and only contribute to one type of response, others such as NK and γδ T cells have a more promiscuous role.

2.1.1.1 Innate immunity

The innate immune system ensures an early response against microorganisms. The first line of defence is provided by surface barriers (e.g. skin, lung and gut epithelia) affording mechanic, chemical and biological protection against pathogens [3]. Microbes that breach these barriers are recognised by pattern recognition receptors (PRR). These are encoded in the germline and can sense a variety of pathogen associated molecular patterns.

PRRs include Nod-like receptors (NLRs), RIG-I like receptors (RLRs) and Toll-like receptors (TLRs). NLRS are a family of 22 cytosolic PRRs that detect PAMPs as well as endogenous molecules (e.g. ATP) and subsequently signal through NF-κB activation [4]. A number of NLRs such as NLR protein 3 (NLRP3) and NLR CARD Domain Containing 4 (NLRC4) are integral parts of the inflammasome, the multi-protein complex that processes the pro-inflammatory cytokines IL-1β and IL-18 into their active form [5]. Mutations of genes encoding subunits of this enzymatic platform lead to monogenic autoinflammatory diseases, as described later on in this chapter

RLRs are a group of cytosolic receptors detecting viral RNA and inducing the production of type-I interferons. They specifically recognise features that are unique to viral genomes (e.g. double-stranded RNA), thus avoiding the initiation of an inflammatory process by endogenous RNA [6].

Toll-like receptors (TLRs) are transmembrane proteins that recognize viral nucleic acids as well as bacterial components, including lipopolysaccharide (LPS) and lipoteichoic acids [7]. More specifically, cell-extrinsic recognition of viruses is mediated by TLR-3, TLR-7, TLR-8 and TLR-9, which are expressed in the endosomes (Fig. 2.1.1.1) [8]. Conversely, TLR-1, TLR-2, TLR-4, TLR-5, TLR-6 and TLR-11 reside on the cell surface and recognize a wide range of PAMPs, such as microbial membrane proteins and parasite components. Following ligand binding, TLR signalling is initiated by association with the Myd88 adaptor molecule. This is followed by a phosphorylation cascade resulting in the activation of the mitogen-activated protein kinase (MAPK) and NF-κB pathways and the transcription of cytokines, chemokines and adhesion molecules. Of note, the same cascade of events is activated downstream of

the IL-36 receptor. This suggests a potential convergence of the two signalling pathways, as explored in the result chapter.

The key mediators of innate immunity are neutrophils, dendritic cells (DC), macrophages and natural killer (NK) cells [1]. These cells can sense pathogens through their PRRs and subsequently produce cytokines, chemokines and anti-microbial molecules.

Neutrophils account for the majority of circulating white blood cells. Following chemokine-driven extra-vasation, they are the first cells to arrive at sites of inflammation, where they are activated by bacterial components such as LPS. Active neutrophils can then amplify the inflammatory response mediated by other cell types, and directly attack microorganisms by phagocytosis [1]. Importantly, the elimination of circulating microbes can also take place through the formation of neutrophil extracellular traps (NETs). NETs are web-like structures constituted of DNA, histones, proteins (e.g. cathepsins) and granule enzymes (e.g. neutrophil elastase). They have been shown to constrict pathogens and enable subsequent phagocytosis [9].

Dendritic cells and macrophages are particularly important for relaying signals between innate and adaptive immunity. In fact, an important feature of these cells is the ability to phagocytise extracellular antigens and load them on class II major histocompatibility complex (MHC) molecules. The antigens are then presented to T helper (Th) lymphocytes, which become activated and initiate an adaptive immune response [1].

Finally, natural killer cells can recognise and kill virus-infected cells and also secrete essential inflammatory mediators such as Interferon-γ and TNF-α.

Despite not being classified as immune cells, keratinocytes (KCs) play an important role in the response to pathogens that have breached the skin barrier. They express a vast array of PRRs, and therefore recognise numerous microbial components. This results in the secretion of defensins and cathelicidin, a group of antimicrobial peptides that can disrupt bacterial cell membranes. KCs also secrete

chemokines (e.g. IL-8), cytokines (e.g. IL-1α) and pro-inflammatory mediators such as CCL20 and MMP9, which activate Langerhans cells (skin resident DCs) and Th cells, respectively [10].

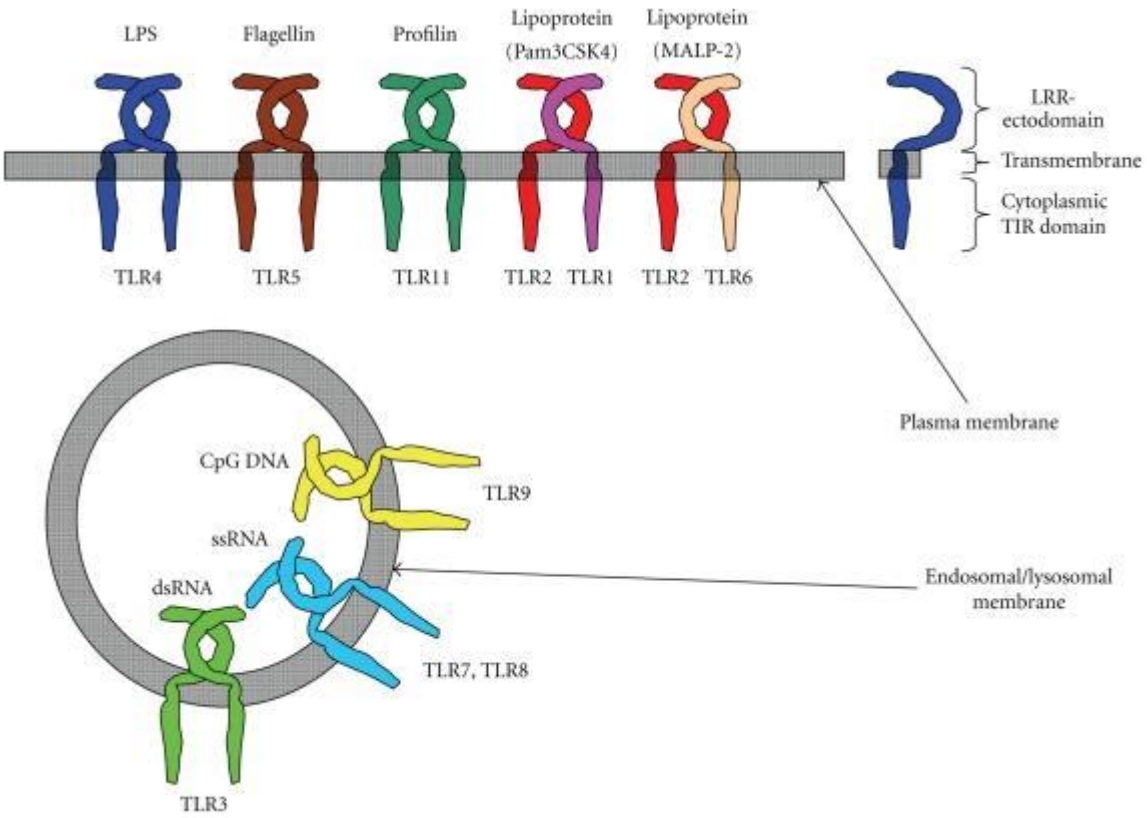


Fig. 2.1.1.1 Toll-like receptors family

The figure from Yamamoto et al. [11] illustrates the key features of Toll-like receptors (TLRs). These transmembrane proteins can be expressed on the plasma membrane (top panel) or in the endosomal (or lysosomal) membrane. TLRs are homo or heterodimers. Each subunit is composed by a cytosolic Toll IL-1 Receptor (TIR) signalling domain, a transmembrane domain and a Leucine-Rich-Repeats (LRR) ectodomain.

2.1.1.2 Autoinflammation

The term autoinflammation refers to an abnormal and seemingly unprovoked activation of the innate immune system, which occurs in the absence of autoantibody production or autoreactive T cells [12]. However, distinguishing between innate and adaptive causes of immune mediated diseases is not always trivial. There is evidence of autoimmunity in syndromes of a predominantly autoinflammatory nature, and innate immune system involvement in autoimmune disease [13]. Therefore, it has been proposed that immune-mediated disorders are distributed along a continuum between autoinflammatory and autoimmune phenotypes (Fig.2.1.1.2).

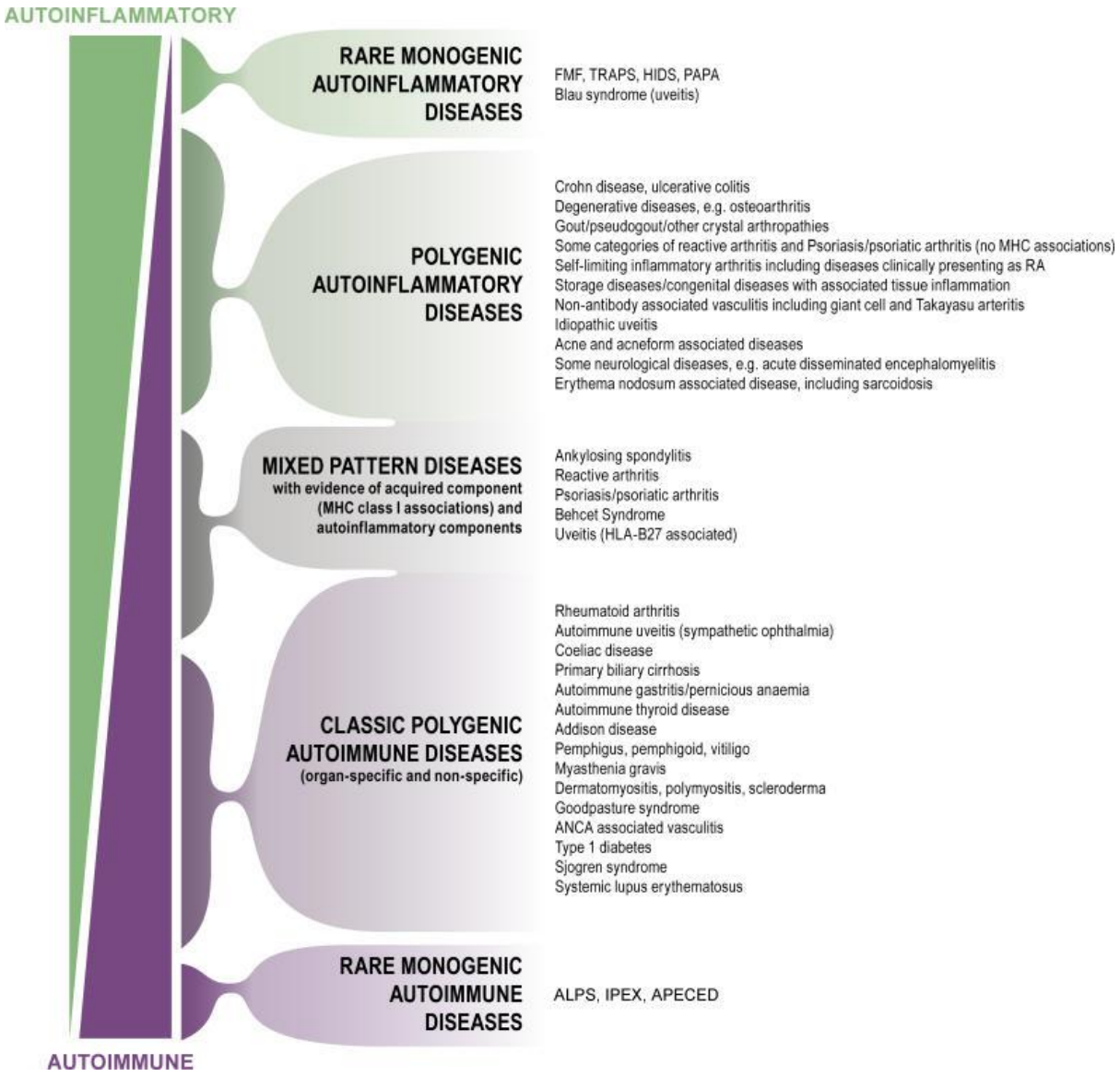


Fig. 2.1.1.2. The immune-mediated disease continuum, with examples.

This representation, created by McGongal and McDermott [13], illustrates how monogenic autoinflammatory and autoimmune diseases represent the extremes of an immunological spectrum. Intermediate phenotypes may manifest with MHC involvement but still present with autoinflammatory features. Psoriasis is a good example of this phenomenon.

Inherited periodic fever syndromes sit at the autoinflammatory end of the spectrum. These Mendelian diseases are mostly caused by autosomal dominant mutations that lead to constitutive innate immune signalling. For example, TNF receptor-associated periodic syndrome (TRAPS), is caused by mutations of the tumour necrosis factor receptor (TNFR1) [12]. Disease alleles result in defective receptor clearance, leading to sustained TNF signalling and prolonged inflammation [14].

Another key mechanism of autoinflammation is excessive IL-1β production. In fact, activating mutations in NLRP3, which is essential for IL-1 processing by the inflammasome, were found in individuals suffering from cryopyrin associated periodic syndromes (CAPS) [15]. These are a group of conditions of varying severity (familial cold autoinflammatory syndrome 1; Muckle-Wells syndrome and neonatal onset multisystem inflammatory disease), that typically present with recurrent fevers, urticarial skin rashes, neutrophilia and arthralgia or arthritis.

The key role played by IL-1 in autoinflammation was confirmed by the discovery of truncating mutations of the interleukin 1 receptor antagonist (IL-1Ra) in individuals presenting with skin pustulation, recurrent fevers and osteomyelitis (Deficiency of Interleukin 1 Receptor Antagonist (DIRA) syndrome) [16]. Since genetic studies demonstrated the key role of IL-1 in the pathogenesis of CAPS and DIRA, significant progress has been made in the treatment of these conditions. In fact, the IL-1 blocker anakinra ameliorates inflammation and its related symptoms in most disease cases [17].

Although the pathogenic potential of innate immune genes has been amply demonstrated, other mechanisms linking conserved cellular functions with autoinflammatory processes have recently emerged [18]. For instance, mutations in genes required for immunoproteasome function (e.g. *PSMB8*, *PSMB9* and *PSMG2*) cause abnormal type I IFN production and sustained inflammation [19]–[21]. As other disease alleles de-regulating type I IFN responses have been identified in *STING*, *IFIH1* and *DDX58*, the term “Interferonopathies” was coined to describe monogenic autoinflammatory disorders associated with excessive type-I IFN production [22]. In keeping with the key pathogenic role

of IFN signalling in these conditions, patients treated with JAK1/2 inhibitors showing beneficial improvements [23].

2.1.1.3 Adaptive immunity

While the innate immune system provides rapid sensing and elimination of pathogens, slower adaptive responses have higher specificity and long-lasting effects (immune memory).

Cells of the adaptive immune system include the T lymphocytes, which mature in the thymus, and the B lymphocytes, which arise in the bone marrow. These cells then traffic to secondary lymphoid organs, including lymph nodes and the spleen, which capture circulating antigens from the lymph and blood, respectively [24].

Naive T cells are activated upon interaction of their T cell receptor (TCR) with antigenic peptides complexed with major histocompatibility complex (MHC) molecules. Peptides produced from proteins translated within the cell are typically MHC class I–restricted (i.e. presented only by human leukocyte antigen (HLA)-A, HLA-B and HLA-C). Conversely, extracellular antigens are generally MHC class II restricted (presented by HLA-DR, HLA-DQ, and HLA-DP) [25]. Unlike MHC class I proteins, which are constitutively expressed in all nucleated cells, MHC class II molecules are only present on antigen presenting cells (APCs) and are inducible by innate immune stimuli, including TLR ligands.

APCs can be found in the skin and mucosal sites, where pathogen encounter is most likely. Of note, keratinocytes can act as APCs by presenting lipid generated by phospholipase A2 to CD1a-reactive T cells. However, this mechanisms is mediated by resident Langerhans cells [26], [27]. Other APCs, instead, migrate to regional lymph nodes, where the interaction with T cells occurs, leading to initiation of immune responses [24], [25].

T cells can be classified into different classes (Fig. 2.1.1.3). The largest group are CD4+ T cells, which have a helper function and are consequentially designated as Th cells. Two main categories of T helper lymphocytes were initially described: Th1 and Th2 [28]. Th1 cells, which differentiate in response to

IL-12 and IFN-γ, are characterized by the production of IFN-γ and IL-2. In contrast, Th2 cells require IL-4 for their development and produce IL-4, IL-5, IL-10, and IL-13.

However, not all CD4+-driven processes could be attributed to cytokines predicted to arise from Th1 or Th2 responses [29]. The discovery of a third Th subset filled this research gap. Th17 cells are induced by IL-6 and TGF-β. They mainly produce IL-17A, IL-17F, IL-21 and IL-22. These are potent proinflammatory cytokines capable of inducing IL-6 and TNF production, as well as driving granulocyte recruitment to damaged tissues [30].

The existence of IL-9–producing Th9 cells has recently been suggested by the observation that exposure of Th2 cells to a combination of IL-4 and TGF-β reprograms them to produce IL-9, a potent mast cell growth factor and mediator of anti-parasite immunity [31], [32].

The regulation of T-cell responses also resides within the CD4+ subset of lymphocytes which, in response to TGF-β and IL-2, acquire the expression of FOXP3, leading to the activation of a new transcriptional repertoire. The change in gene expression allows this specialised cell-type, called T regulatory (T regs) cells, to suppress immune responses, thereby maintaining homeostasis and self-tolerance [33].

The second largest group of T cells, CD8+ T cells, acts to remove viruses and transformed cells. CD8+ T cells are primarily activated by antigenic peptides presented on MHC class I molecules. Recognition of these cytosolic peptides then leads to apoptotic death of the target cell. This process is mediated by rapid mobilization of CD8+ T cell granules and exocytosis of their contents, including granzymes and perforin [34].

Natural killer T (NKT) cells represent another T cell subset, which recognize non-peptide antigens presented by non-classical MHC molecules of the CD1 family. Activated NKT cells are capable of rapid and substantial production of cytokines, including IL-4, and have been implicated in allergic pathogenesis [35].

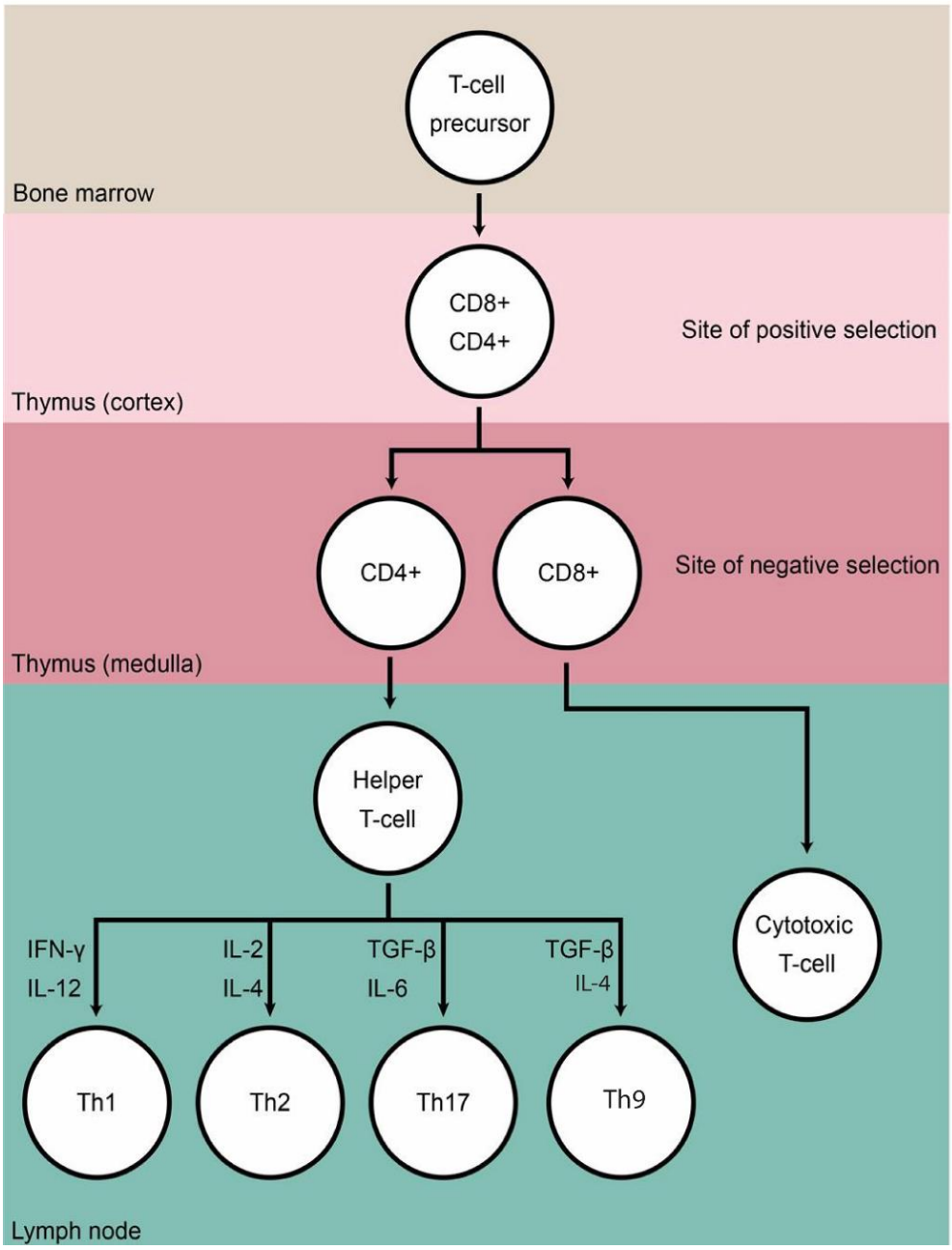


Fig. 2.1.1.3. T cells phenotypes.

The diagram (adapted from www.step1.medbullets.com) shows the development of T cells from the bone marrow, where they originate, to the thymus, where they differentiate into $CD4^+$ or $CD8^+$ subsets. The latter migrate to secondary lymphoid organs where they become polarises towards different phenotypes, such as Th1/2 and Th17, in response to specific cytokines.

Adaptive humoral immunity is mediated by antibodies produced by fully differentiated B cells (also known as plasma cells). These arise from haemopoietic stem cells in the bone marrow, where commitment to the B-cell lineage is under the control of several transcription factors, such as PU.1, IKAROS (IKAROS family zinc finger 1), E2A, EBF (early B cell factor 1), PAX5 (paired box gene 5) and IRF8 (interferon regulatory factor 8) [36], [37]. Once they have acquired antigen specificity and left the bone marrow, immature B cells complete their development to the mature naive stage. This requires the expression of IgD, as well as IgM, on the cell surface. The entire process occurs in the absence of any contact with exogenous antigens. Thus it is known as antigen-independent B-cell development.

The second phase of B-cell development occurs after the encounter with an antigen and is therefore called the antigen-dependent activation phase. This requires two signals. The first one is immunoglobulin receptor cross-linking. The second is the interaction between B and T cells. When the B cell contacts a CD4+ T cell that is specific for the peptide presented on the B cell-MHC class II molecule, the T cell is able to activate the B cell for further differentiation. Depending on the various contacts and cytokine stimuli received by the activated B cell, it will become either a memory cell or a plasma cell producing large amounts of antibody (Fig. 2.1.1.3.1).

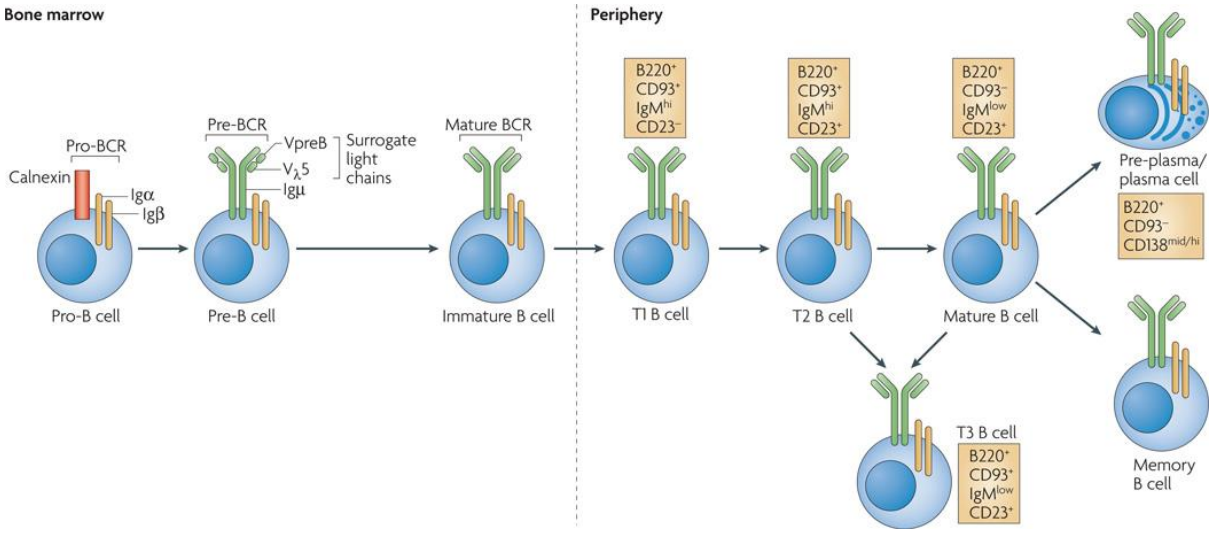


Fig. 2.1.1.3.1. B cell development.

This figure from Cambier et al. [38] illustrates how B-cell development occurs in the bone marrow and in peripheral lymphoid tissues such as the spleen. In the bone marrow, development progresses up to the immature B cell stage via sequential immunoglobulin re-arrangements. B cells then undergo a selection process to prevent any further development of self-reactive cells. Cells that have passed this checkpoint leave the bone marrow as transitional B cells, eventually maturing into memory B cells or antibody secreting plasma cells.

2.1.1.4 Autoimmunity

Autoimmunity is a response of the immune system against self-antigens. Under normal circumstances, adaptive immune cells and APCs undergo a process of "education" in the germinal centre, so they can discriminate between host (self) and microbial (non-self) proteins. When the tolerance to self is broken, the organism starts to produce auto-antibodies and auto-reactive T cells, which lead to the development of autoimmune diseases.

The causes of autoimmunity are thought to be an interplay between genetic and environmental factors. In this context, genome-wide association studies (GWAS) have identified very significant HLA associations (e.g. HLA-DQA1*0501 in celiac disease or HLA-DRB1*1501 in multiple sclerosis) [39]. While these tend to be disease specific, several susceptibility genes that contribute to multiple autoimmune diseases have also been identified [40]–[42]. These include various genes that mediate the effects of IL-23 on the polarization of Th17 cells [43]. For example genetic polymorphisms in IL23R (which encodes a subunit of the IL-23 receptor) have been associated with ankylosing spondylitis, Behcet's disease, Crohn's disease, psoriasis, and ulcerative colitis [44]. Of note, targeting the IL-23/Th17 axis with monoclonal antibodies specific for either p40 (a subunit of IL-23) or IL-17A has shown efficacy in many of these disorders [45], [46].

While autoimmune diseases are often described as complex polygenic disorders, disruption of a single gene can in some cases result in breach of self-tolerance. Perhaps the two best known examples of this phenomenon are autoimmune polyendocrine syndrome (APS) and immunodysregulation polyendocrinopathy enteropathy X-linked (IPEX) syndrome. These diseases result from mutations in AIRE and FOXP3, respectively, leading to catastrophic dysfunction in central (APS) and peripheral (IPEX) tolerance [47], [48].

2.2 IL-36 cytokines

2.2.1 Overview

The term interleukin (IL)-36 refers to a group of IL-1 family cytokines that bind a common heterodimeric receptor (IL-36R). These ligands include three pro-inflammatory proteins (IL-36α/IL-1F6, IL-36β/IL-1F8 and IL-36γ/IL-1F9) and a receptor antagonist (IL-36Ra/IL-1F5).

The IL-36 receptor is composed of a signalling subunit (also known as IL-1Rrp2 and encoded by the *IL1RL2* gene) and an accessory protein (IL-1RAcP, encoded by *IL1RAP*). While IL-1Rrp2 only binds IL-36 cytokines, IL-1RAcP is also a component of the IL-18 and IL-33 receptors [49].

2.2.2 Expression and processing of IL-36

The genes encoding IL-36 cytokines are clustered on human chromosome 2q14 together with those for all other IL-1 family cytokines (except for *IL18* and *IL33*, which map to chromosome 11p14 and 9p24, respectively) [50].

Like all other IL-1 family members (except for IL-1Ra), IL-36 cytokines are produced as inactive precursors that require proteolytic processing for activation (Fig. 2.2.1). In fact, Towne and colleagues have found that removal of a small number of residues from the N-termini of IL-36α, IL-36β and IL-36γ (IL-36) increases their biological activity by greater than 10 000-fold [51]. Clancy et al. have built on these data to show that IL-36 can be processed by caspase-3 with high specificity. Others have demonstrated that cathepsin S, which is up-regulated in skin inflammation, can also cleave IL-36 [52]. Finally, elastase, a protein produced by neutrophils during inflammation, has been identified as a proteinase that specifically activates IL-36γ and IL-36Ra [53], [54].

The IL-36 cytokines are expressed in a variety of cell types, with abundant transcript levels observed in keratinocytes, bronchial epithelium, gut mucosa, neurons, glial cells, dendritic cells (DCs) and macrophages. In most of these cell types, IL-36 levels are further increased in response to inflammatory stimuli, including cytokines (e.g. IL-1), microbial infections (e.g. *Aspergillus fumigatus*

[55]) and cigarette smoke [56], [57]. *IL1RL2*, on the other hand, is widely expressed. Transcript levels are typically low and do not seem to be tightly regulated [58], [59].

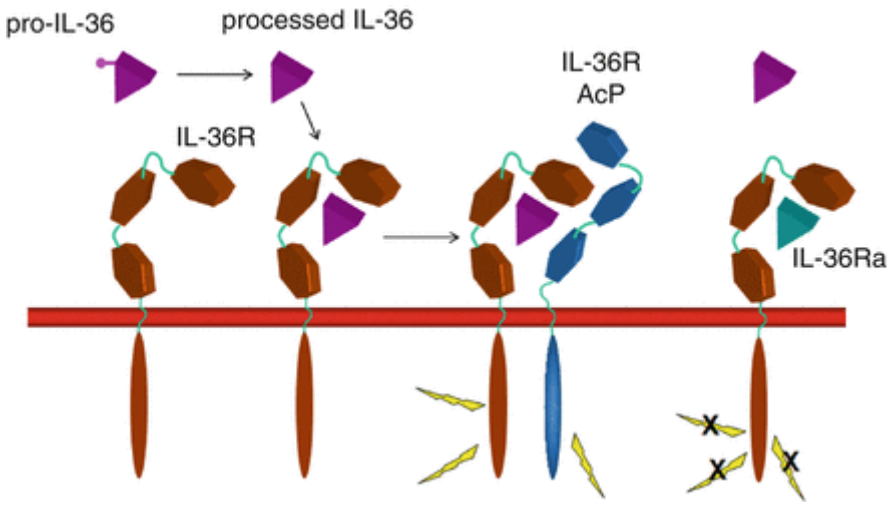


Fig. 2.2.1. Overview of IL-36 signalling.

Interleukin (IL)-36 is originally synthesised as an inactive precursor (pro-IL36) that requires N-terminal processing. Once IL-36 has bound its receptor (IL-1Rrp2), a heterodimeric complex is formed which is capable of signalling. IL-36Ra antagonizes this process by binding to IL-36R in a fashion that blocks the recruitment IL-1R AcP and therefore inhibits downstream signalling. Figure from Sims et al [60].

2.2.3 IL-36R signal transduction

IL-36R signalling shares common features with IL-1R signal transduction. Both receptors are heterodimers and share the same accessory protein (IL-1RAcP). The latter is composed of an extracellular ligand-binding domain, a single-pass transmembrane sequence, and a cytoplasmic Toll-IL-1 receptor (TIR) domain. The binding of the agonists stabilises the accessory protein, which induces conformational changes in the TIR domain. The protein complex then recruits the adaptor protein MyD88, IL-1 receptor-associated kinase 1 (IRAK-1), and the Toll-interacting protein (Tollip), leading to the activation of transcription factors such as NF-κβ and IκBz. (Fig. 2.2.2). This in turn leads to the expression of innate immune cytokines (e.g. IL-6 and IL-8) [59], chemokines (CCL3, CCL4, CCL20) [61], anti-microbial peptides (e.g. LL37) and matrix metalloproteinases [62]. While other pro-inflammatory mediators are likely to be up-regulated by IL-36, most studies investigating its effects were based on the use of unprocessed molecules with minimal biological activity. Thus, our understanding of IL-36 target genes is incomplete.

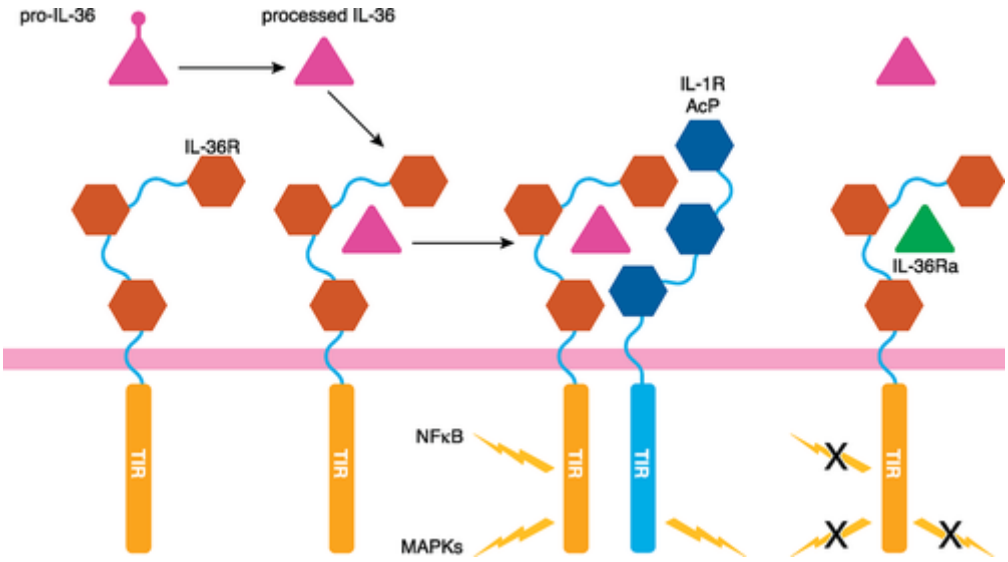


Fig. 2.2.2. **IL-36R activation and downstream signalling cascade.**

Once IL-36 precursors have been processed, they bind to IL-36R, which associates with IL-1RAcP. The first downstream event is then activation of the TIR, which in turn triggers NF-ķB and MAPK signal transduction. Figure from Sims et al [60].

Despite other similarities with IL-1R, IL-36R intracellular trafficking follows a distinct pattern. *In-vitro* experiments by Saha and colleagues showed that in the absence of an antagonist, IL-36R cycles into endosomes and back to the plasma membrane. Exposure of HaCat cells to IL-36 alters the dynamics of this process, so that an increased proportion of the IL-36R enters the lysosomes where it is degraded. IL-36 also increases the co-localization of Tollip and IL-36R in the endosome. These results suggest that Tollip is involved in IL-36R trafficking and that the presence of agonists acts as a feedback mechanism, regulating the availability of receptor for downstream signalling [63].

2.2.4 The immune function of IL-36

2.2.4.1 IL-36 and response to pathogens

Like other cytokines of the IL-1 family, IL-36 contributes to the response against pathogens that invade barrier tissues, such as skin, gut and lungs.

The role of IL-36 in the defence against *Aspergillus fumigatus* and *Candida albicans* is well established [55], [64]. In fact, PBMCs exposed to *A.fumigatus* produce IL-36γ in a TLR-4 dependent fashion [55]. Moreover, IL-36Ra treatment reduced *A.fumigatus* (or *C.albicans*) dependent Th17 polarization and consequent IL-17 production [64], [65].

Accumulating evidence also supports a role of IL-36 in immunity in tuberculosis. IL-36R knockout mice, for example, produce less Th1 cytokines (IFNγ, TNFα and IL-6) than wild type littermates, when infected with *Mycobacterium bovis Bacillus Calmette-Guérin* (BCG) [66]. The pulmonary lesions of IL-36R deficient mice were also more extensive than those of wild type animals, indicating that IL-36 mediated immune responses protect from damage induced by infection. Additionally, recent *in-vitro* studies demonstrated that *Mycobacterium tuberculosis* can induce IL-36γ production in human macrophages, by activating TLR2/4-MyD88 signalling [67].

2.2.4.2 Effects of IL-36 on immune cells

Despite the distinction between adaptive and innate immunity, their interplay is essential for an efficient response to infections. In this context, accumulating evidence suggests that IL-36 has a key role in the cross-talk between antigen presenting cells and T lymphocytes, leading to the polarization and activation of the latter cell type.

Vigne et al. first showed that in murine myeloid dendritic cells (mDCs), IL-36 upregulates activation markers such as CD80, CD86 and MHC class II molecules. It also induces IL-6 and IL-12 production [68]. Moreover, stimulation of murine $CD11^+$ cells with IL-36α upregulates CXCL1 and CXCL2, which are potent neutrophil chemoattractant [69].

In humans, abundant IL-36R expression has been reported in myeloid DCs, including monocyte-derived DCs (MDDCs). The latter respond to IL-36β and IL-36γ by up-regulating activation markers (HLA-DR and CD83) and cytokine (IL-12 and IL-18) release [70].

Vigne et al also showed that IL-36 is highly expressed in naïve $CD4^+$ T cells and synergizes with IL-2 in driving Th1 polarization [65]. Furthermore, Harusato et al. reported that IL-36 activates MyD88-NF-ķB in $CD4^+$ T cells to potently inhibit the development of Foxp3-expressing regulatory T cells (Treg) [71]. Concomitantly, IL-36 can also influence Th9 polarization through IL-2-STAT5- and IL-4-STAT6-dependent pathways. Thus, IL-36 seems to play a key role in Th9-Treg balance that, if disrupted, can lead to intestinal inflammation and associated chronic disorders [71].

It is important to bear in mind, however, that most of these studies were carried out in mice, so that the results may not apply to human T cells. It is also unclear whether the above observations reflect a direct effect of IL-36 on T cells, given that the receptor is not detectable on the surface of human, circulating T lymphocytes [61], [70].

2.2.4.3 Pathogenic effects of IL-36

While IL-36 plays an important physiological role in anti-fungal and anti-mycobacterial defences, accumulating evidence indicates that it can also have pathogenic effects, if produced in excessive amounts (Fig.2.2.3).

Since the expression of IL-36 cytokines is especially prominent in keratinocytes, it is not surprising that most studies have focused on skin disorders. In particular, IL-36 has been repeatedly investigated in the context of psoriasis.

Several reports show that psoriatic skin lesions over-express IL-36R ligands [62], [72]–[74]. This is supported by the observation of skin acanthosis and hyperkeratosis (which are key characteristics of psoriatic lesions) in transgenic mice over-expressing IL-36α [38]. Importantly, IL-36Ra deficiency exacerbates the phenotype of IL-36α transgenic mice, suggesting that IL-36Ra antagonises IL-36 mediated skin inflammation *in-vivo* [72].

There have also been reports of an association between psoriatic arthritis and SNPs mapping to the *IL36B/IL36RN* gene region [75]. Moreover, our group and others have demonstrated that mutations in *IL36RN* cause generalised pustular psoriasis (GPP), a rare and potentially life-threatening disease manifesting with flares of neutrophilic skin inflammation [76]–[80] (see section 5.7).

IL-36 has also pro-inflammatory effects beyond skin. IL-36α, for instance, is expressed by adipocytes and M2 macrophages present in adipose tissue during inflammation [81].

In the airways, IL-36α administration up-regulates the murine CXCL1 and CXCL2 chemokines, promoting an influx of neutrophils in the lungs. A similar phenomenon is observed in the nasal epithelial cells of patients with chronic rhinosinusitis, where abundant IL-36γ expression leads to neutrophil infiltration and activation [53].

In inflamed synovial tissue, especially in patients with psoriatic or rheumatoid arthritis, infiltrated $CD138^{+}$ plasma cells actively produce IL-36α. In keeping with these findings, serum IL-36 levels correlate with disease activity [82], [83].

In dextran-sulphate sodium (DSS) induced ulcerative colitis, IL-36 plays a critical role in driving the recruitment of immune cells (especially macrophages and neutrophils) to the lamina propria [84]. In fact, *Il1rl2* knockout mice suffer from less severe chronic colitis and intestinal fibrosis, following DSS administration [85]. In keeping with these findings, the expression of IL-36α is elevated in the colonic mucosa of ulcerative colitis patients [84].

Finally, local IL-36α over-expression in the kidney is associated with tubule-interstitial lesions in mouse models of chronic glomerulonephritis, systemic lupus erythematosus (SLE), nephrotic syndrome and diabetes [86]. However, the clinical significance of IL-36 up-regulation in these diseases remains to be elucidated.

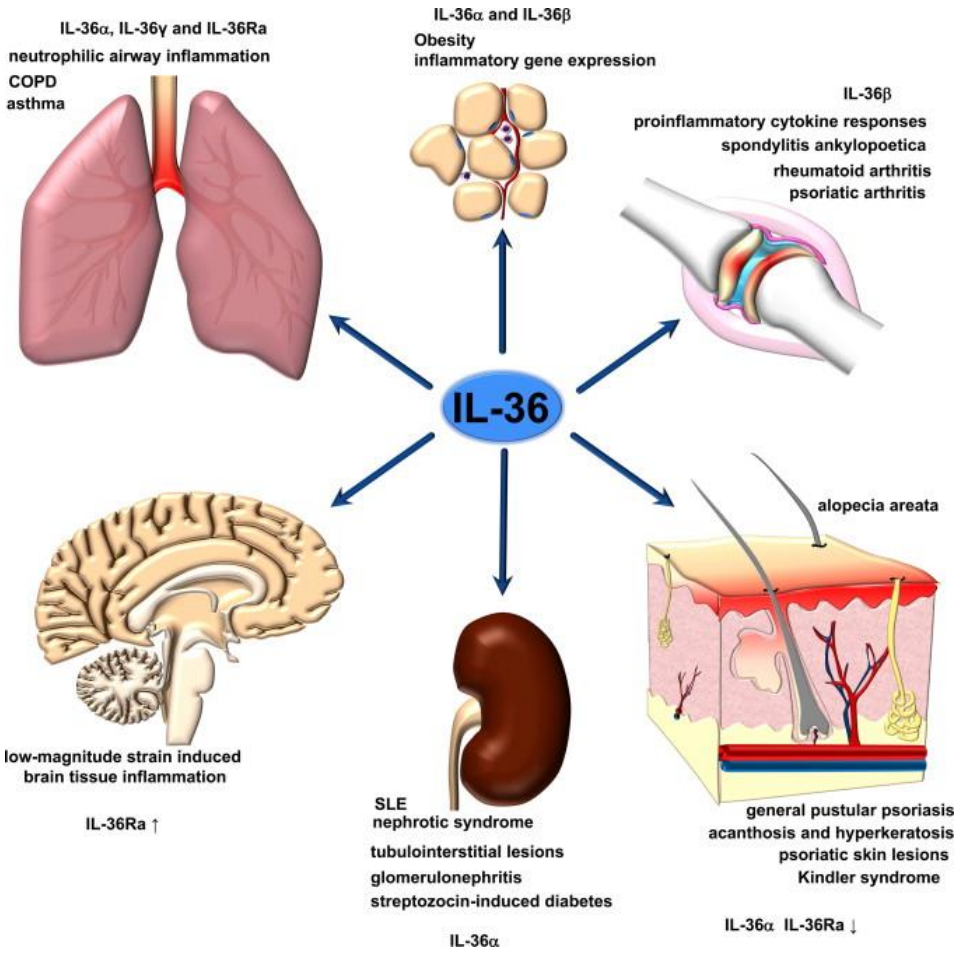


Fig. 2.2.3. **Effect of IL-36 on different tissues and tissue-specific disorders.**

IL-36 cytokines are key mediators of inflammation in several tissues. The diagram shows the IL-36 cytokines that have been implicated in the pathogenesis of organ-specific inflammatory diseases. Adapted from Gresnigt MS et al.[49].

2.3 Psoriasis

Psoriasis is a chronic, immune-mediated skin disorder with a prevalence of 2-3% in populations of European descent. Males and females are equally affected and the disease is often associated with comorbidities, such as psoriatic arthritis [87]–[89].

Psoriasis can be classified in different forms, based on the nature and localization of skin lesions (Table 2.3, Fig. 2.3.1). Given that the focus of this thesis is on plaque and generalised pustular psoriasis, these clinical variants will be discussed in more detail.

Table.2.3. Classification of psoriasis clinical variants

Type of psoriasis	Most affected areas	Characteristic lesions
Plaque	Scalp, elbows, trunk	Plaques covered with white scales
Pustular	Widespread lesions (generalised pustular psoriasis); palms and soles (palmar plantar pustlosis)	Sterile pustules
Guttate	Trunk and limbs	Plaques with a fine adherent scale
Erythrodermic	> 90% of total body surface	Generalised erythema and scaling
Inverse	Skin folds	Plaques with minimal scale
Nail	Finger nails or toenails	Subungual hyperkeratosis

Fig. 2.3.1. **Classification of psoriasis.**

Examples of the different forms of psoriasis described in Table 2.3. From the top-left: plaque, guttate, pustular and erythrodermic psoriasis. Images from Hon Pak, M.D. published on eMedicine.net.

2.3.1 Plaque Psoriasis

2.3.1.1 Clinical presentation

Plaque psoriasis (Ps, also known as Psoriasis Vulgaris (PsV)) is the most common form of psoriasis, accounting for >90% of disease cases [90].

It classically presents with well demarcated, erythematous plaques with loosely adherent silvery scales. These lesions have a symmetrical distribution and most commonly affect the scalp or extensor surfaces, such as elbows and knees.

The physical burden of the disease is increased by co-morbid conditions, such as seronegative psoriatic arthritis, which affects up to 30% of patients [8]. Several observational studies have also identified an elevated cardiovascular risk among patients with severe psoriasis [9]–[11].

At the histological level [12], psoriatic plaques are characterised by the presence of hyperproliferative keratinocytes that retain their nuclei, even in the uppermost layers of the epidermis (parakeratosis). This is due to incomplete terminal differentiation, as demonstrated by reduced expression of keratin 10.

T lymphocytes and dendritic cells (DC) densely infiltrate psoriatic lesions. Neutrophils also accumulate in 'Kogoj pustules' and 'Munro's microabscesses', which appear in the stratum spinosum and stratum corneum of the epidermis, respectively. Finally, the erythematous appearance of lesions is due to dilated and tortuous blood vessels, which reach into the tips of elongated dermal papillae.

2.3.1.2 Genetics

Epidemiological surveys have long demonstrated that Ps is more frequent among the relatives of affected individuals compared to the general population [91]. Moreover, a higher disease concordance has been consistently observed in monozygotic compared to dizygotic twins, with heritability estimates ranging from 60 to 90% [92]. As a result, Ps is widely considered as a multifactorial trait, mediated by an interplay between inherited susceptibility alleles and environmental factors.

Linkage studies carried out in the 1990s identified various loci co-segregating with the disease in multiplex pedigrees. The most reproducible signal was observed in the so-called *PSORS1* region, which maps to the class I interval of the major histocompatibility complex (MHC) and explains 35-50% of disease heritability [93]–[95]. The linkage signal is driven by the *HLA- Cw*0602* allele, which has been repeatedly described as the strongest genetic determinant of the disease [96]–[98].

Disease associated variants have also been identified within the *PSORS2* locus on chromosome 17q25 and, more specifically, in the *CARD14* gene, which encodes a nuclear factor-κB (NF-κB) activator. Jordan et al first reported deleterious *CARD14* mutations in extended pedigrees presenting with rare monogenic forms of Ps [99]. Further studies then showed that common *CARD14* alleles are also associated with the disease in the general population [100].

With the advent of genome wide association studies (GWAS) >40 additional psoriasis susceptibility loci have been identified. These include genes involved in antigen presentation (*ERAP1*), Th17 cell activation (e.g. *IL23R, IL23A, IL12B, TRAF3IP2*) and skin barrier function (*LCE3B/3D*) [101]–[104]. The analysis of targeted genotyping arrays, such as the Immunochip and exome chip, uncovered further susceptibility loci and further confirmed the role of the IL-23/Th17 axis in disease pathogenesis [100], [105], [106].

Despite these achievements, only 5% of lead SNPs described to date are likely to be causal [107] and less than 25% of disease heritability is accounted for by known loci [108]–[110]. There is therefore a

need for further genetic studies, including the fine mapping of susceptibility intervals and the analysis of non-European datasets [111].

2.3.1.3 Immunopathogenesis

Psoriasis can be initiated by trauma, virus infections and certain drugs (such as recombinant IFN-α) [112]. Interestingly, all these triggers cause keratinocytes to secrete cathelicidin/LL37. This is a small antimicrobial peptide that can bind self-RNA or self-DNA released by dying cells. The resulting complexes breach tolerance to self-nucleic acids and activate plasmacytoid dendritic cells (pDC) via Toll-like receptor (TLR)-9 [113]. Myeloid dendritic cells (mDC) are subsequently activated by pDC-derived type I interferon or through a direct interaction between TLR-8 and LL37/RNA complexes [114].

mDCs then migrate to sites of inflammation where they induce Th17 polarization and IL-17 production [115].

In skin, IL-17 acts mainly on keratinocytes, where it induces the transcription of chemokines that attract further DCs and Th17 cells (e.g. CCL20), as well as neutrophils (IL-8) [116], [117]. Thus, IL-17 molecules produced by Th17 cells activate a positive feedback loop, leading to sustained cytokine secretion and chronic inflammation (Fig. 2.3.2).

Given the critical role of T cell activation in disease pathogenesis, the existence of Ps auto-antigens presented by mDCs has been hypothesized. In fact, recent research has demonstrated that peptides derived from LL37 may be recognised by T cells in a *HLA-Cw*0602* restricted manner [118]–[120]. Other studies have highlighted a potential role of lipid antigens generated by phospholipase A2 and presented by skin-resident Langerhans cells [26], [27], [121].

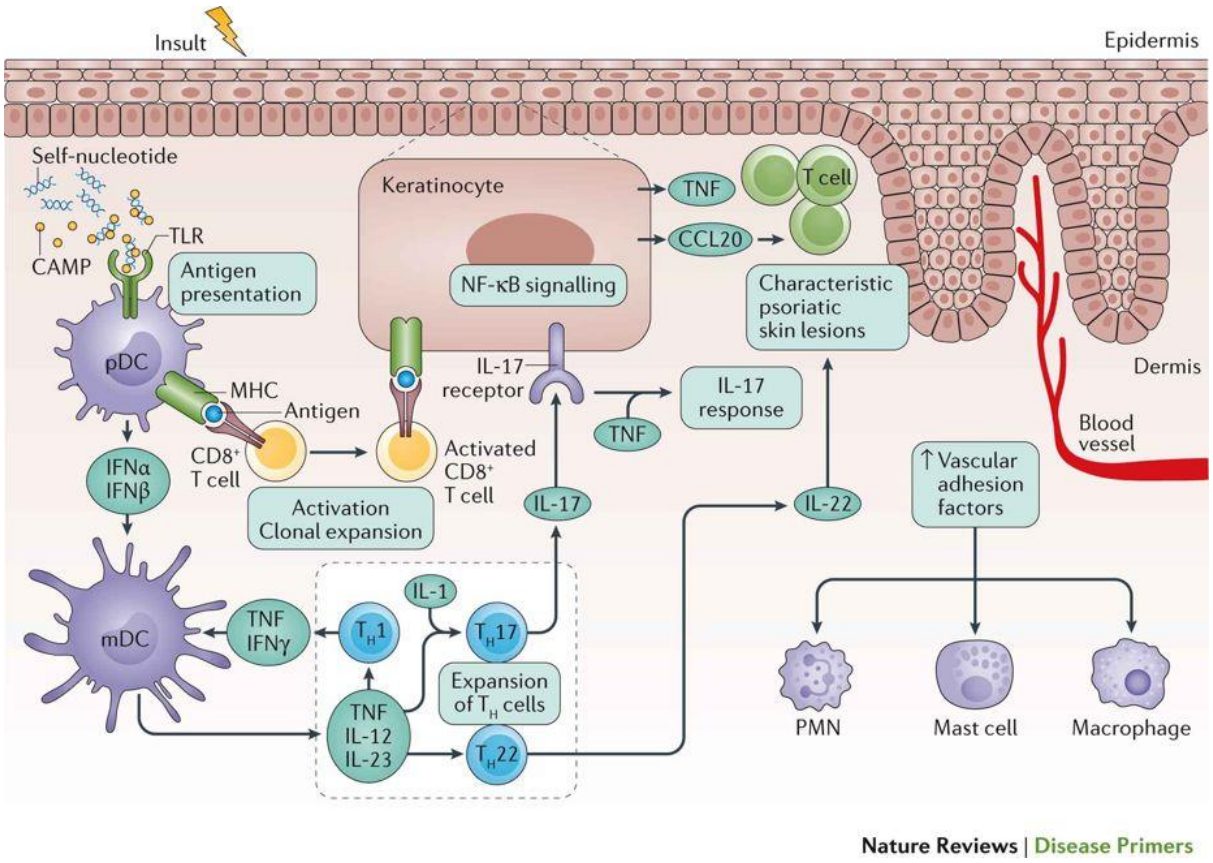


Fig. 2.3.2. Current understanding of plaque psoriasis pathogenesis

The diagram from Greb et al.[122] illustrates the complex pathogenic cross-talk between immune cells and keratinocytes. Following trauma or infection, activated keratinocytes produce LL37 (CAMP), which up-regulates type-I IFN production in pDCs. The resulting inflammatory response is amplified by mDCs through the secretion of IL-23 and subsequent polarization of Th17 cells. These release IL-17 which acts on keratinocytes, thus establishing a pro-inflammatory feedback loop.

2.3.1.4 Treatment

The therapeutic approaches used to treat psoriatic patients vary, based on the severity of the disease and the presence of comorbidities. Topical agents remain the first-line therapy for patients with mild-to-moderate psoriasis. They can be used as standalone treatment or in combination with phototherapy, which suppresses Th17 differentiation and promotes activation of regulatory T cells [123], [124].

For individuals affected by severe disease the use of biologics (monoclonal antibodies that target specific inflammatory cytokines) is recommended, as these agents have shown great therapeutic efficacy.

TNF-α antagonists (adalimumab, etanercept and infliximab) were the first biologics used for the treatment of Ps. They suppress Th17 differentiation by inhibiting IL-23 production by mDCs [125], [126]. Ustekinumab has also been used in the clinic for several years. It is an inhibitor of the common subunit shared by IL-12 and IL-23 (p40) and antagonises both Th1 and Th17 function [46], [127], [128].

More recently, the results of GWAS have informed the development of new agents that selectively target IL-17. These include secukinumab and ixekizumab, two IL-17A monoclonal antibodies, which have shown superior efficacy to ustekinumab [129]–[131]. While the efficacy of IL-17 antagonists supports the role of IL-17 as a key disease driver, this cytokine is also important for anti-microbial immunity. In fact, increased rates of *Candida* infections have been reported after pharmacological IL-17 blockade [130], [131].

Following the success of ustekinumab, selective IL-23 blockers are also being developed. Two agents (Risankizumab and Guselkumab) have already been approved, as they have demonstrated rapid and sustained clinical improvements, alongside an attenuation of molecular signatures of the disease [132].

Finally, Janus kinase (JAK) inhibitors are a new class of small molecule inhibitors, which act on the tyrosine kinases that activate STAT proteins (Fig. 2.3.3). Tofacitinib (a JAK-1/-3 inhibitor) demonstrated non-inferiority to etanercept in a phase III clinical trial, with sustained efficacy over 1 year [133], [134]. Encouraging results were also obtained in a phase II clinical trial of a TYK2 inhibitor [134]. Interestingly, the study's participants were not responding to other treatments, so that selective TYK2 inhibition could offer an alternative for the treatment of mild to severe psoriasis.

In conclusion, advances in characterising molecular disease mechanisms have successfully translated into the development of targeted immunotherapies for plaque psoriasis. However, the current pathogenic models are not sufficiently sophisticated to account for the heterogeneity of the disease and the manifestation of comorbidities. Hence, there is still a need to refine our understanding of Ps.

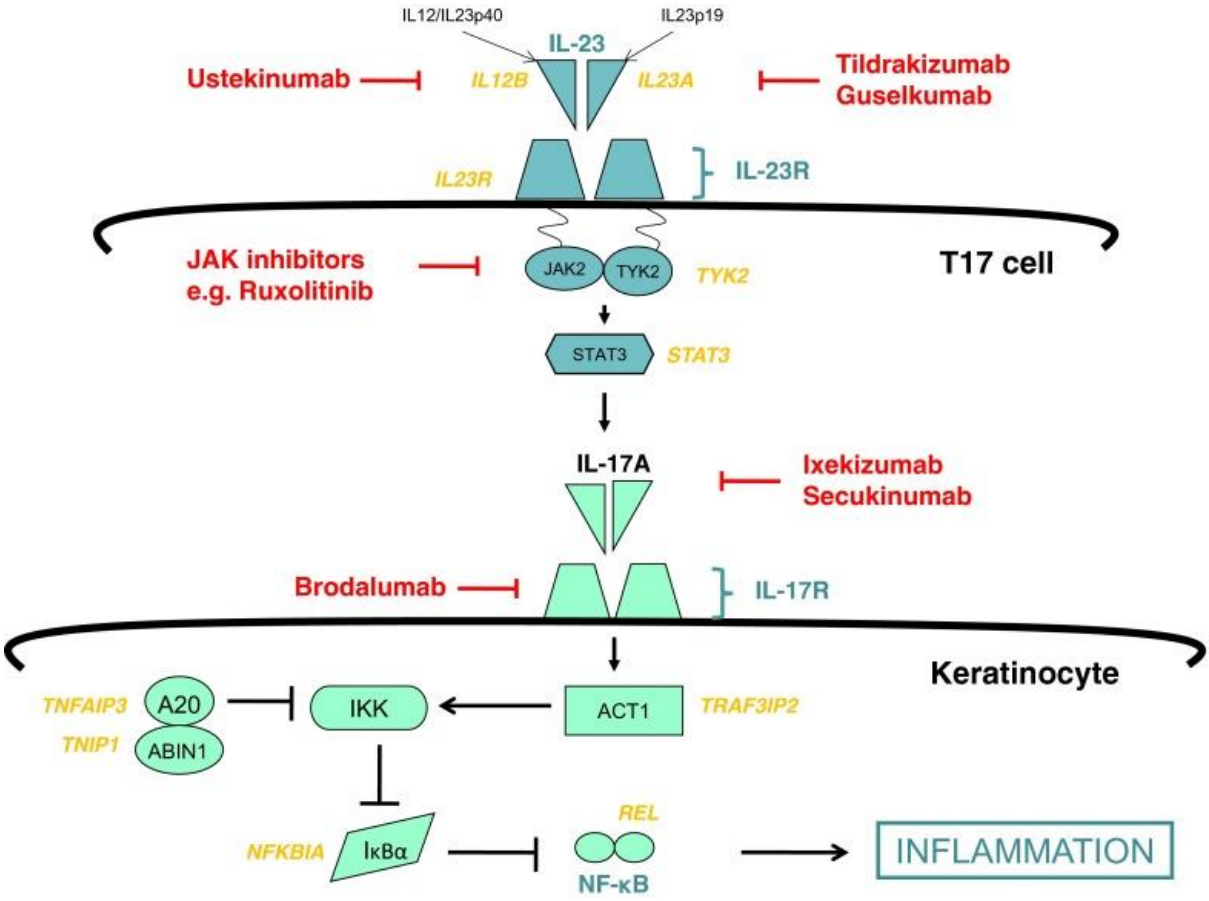


Fig. 2.3.3. **Psoriasis targeted immunotherapy.**

The diagram from Mahil et al [135], illustrates the molecules that can be targeted in order to treat plaque psoriasis. The most effective drugs target the IL-23/Th17 axis. They inhibit IL-23 signalling by competing with the binding of the cytokine to its receptor (Ustekinumab, Tildrakisumab and Guselkumab) or by blocking signal transduction downstream of IL-23R activation (JAK inhibitors). Several drugs have also been developed to inhibit Th17 activity by targeting IL-17 binding to its receptor (Ixekizumab and Secukinumab) or blocking IL-17R itself (Brodalumab). The genes highlighted in yellow have all been identified as psoriasis susceptibility loci in GWAS, demonstrating the power of genetic studies to uncover critical pathogenic pathways.

2.3.2 Generalised Pustular Psoriasis

2.3.2.1 Clinical presentation

The term pustular psoriasis refers to a group of inflammatory disorders presenting with the eruption of sterile pustules on erythematous skin. These are painful lesions, that are histologically characterised by diffuse infiltration of neutrophils in the dermis and the epidermis [136], [137] (Fig. 2.3.2.1).

While pustular forms of psoriasis often have a chronic localised course (palmar plantar pustulosis, acrodermatitis continua of Hallopeau), an acute generalised form is also well recognised.

Generalised pustular psoriasis (GPP) is a very rare (prevalence 1-9:1,000,000), but potentially life-threatening condition. It is characterised by acute episodes of pustulation and widespread erythema, which are often complicated by systemic upset (high fever, increased levels of acute phase reactants, neutrophilia).

According to the consensus diagnostic criteria agreed by the European Rare and Severe Psoriasis Expert Network (ERASPEN), the pustules have to be sterile and macroscopically visible [138]. In fact, very small pustules can appear on the edges of plaques, in patients suffering from severe and unstable Ps. This phenotype, however, does not meet the criteria for a GPP diagnosis and is considered a manifestation of plaque psoriasis [139].

Another condition confounding the diagnosis of GPP is acute generalised exanthematous pustulosis (AGEP). This is an adverse reaction to certain drugs (antibiotics, anticonvulsants, antihypertensive, antipyretics, chemotherapeutics, antifungal and antimalarial agents), manifesting with the abrupt onset of pustular eruptions. AGEP episodes, however, tend to be shorter than GPP flares and are usually self-limiting. Given that AGEP does not recur in the absence of the culprit drug, a history of repeated pustular eruptions is usually sufficient to discriminate between GPP and AGEP [140], [141].

GPP flares can be triggered by pregnancy, infection and certain drugs [142]. The mean age of onset is ~30 years and females are more frequently affected than males [143]. Of note, up to 30% of GPP

patients present with concomitant plaque psoriasis [144], suggesting the existence of shared pathogenic pathways.

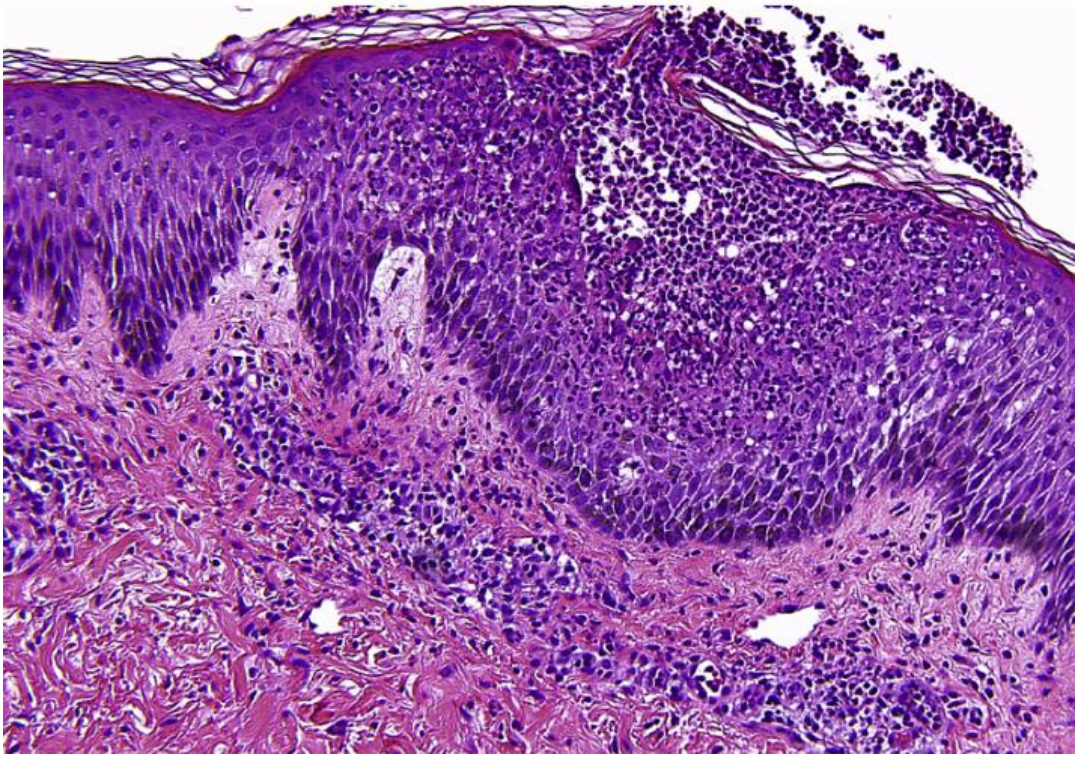


Fig. 2.3.2.1. Histology of pustular psoriasis.

Histology of inflamed skin showing neutrophilic pustules and acanthosis. Haematoxylin-eosin stain; original magnification: ×20. Adapted from [145].

2.3.2.2 Genetics

Pustular psoriasis has a distinct genetic architecture to plaque psoriasis, which is underscored by a lack of association with the *PSORS1* locus [146]. Indeed, the severity and rarity of the clinical phenotype suggested the involvement of rare and deleterious disease alleles, rather than common variants of small effect.

This model was validated by the discovery of recessive GPP mutations in the gene encoding the IL-36 receptor antagonist (*IL36RN)* [77]. Given the phenotypic similarities with the deficiency of the interleukin-1 receptor antagonist (DIRA, an autoinflammatory condition presenting with early onset generalized pustulosis, multifocal osteomyelitis, and high levels of acute-phase reactants [147]), the acronym DITRA (deficiency of interleukin thirty-six-receptor antagonist) has been subsequently proposed to describe generalised pustular psoriasis resulting from *IL36RN* mutations [76].

Follow-up studies have demonstrated that *IL36RN* disease alleles are the strongest genetic determinant of GPP in all examined ethnic groups. In populations of European descent, p.Ser113Leu is the by far the most frequent change, whereas c.115+6T>C and p.Pro27Leu founder alleles dominate in Asian and North-African cohorts, respectively [76], [77], [148].

On the whole, *IL36RN* mutations are found in ~25% of GPP cases. While these disease alleles were originally described as recessive, individuals harbouring a single heterozygous change have also been observed [143]. Interestingly, the onset of the disease is delayed in patients who carry monoallelic *IL36RN* changes [143].

When *IL36RN* is mutated, sustained IL-36 signalling leads to enhanced NF-κB and MAPK activation, with abnormal production of innate pro-inflammatory cytokines (Fig. 2.3.2.2). In fact, *ex-vivo* IL-36 stimulation of patient PBMCs (peripheral-blood mononuclear cells) leads to excessive release of IL-1α, IL-6, IL-8 and TNF[77]. Likewise, patient-derived keratinocytes produce high levels of IL-8 after IL-36, IL-1β and poly(I:C) treatment [76].

Importantly, *IL36RN* mutations do not contribute to Ps susceptibility, as screening of 349 unrelated cases did not identify any enrichment for damaging changes in the IL-36 receptor antagonist [149]. Likewise, genome-wide association studies did not detect any Ps associated alleles within the *IL36RN* gene region [65].

While IL-36 is up-regulated in Ps skin, these observations suggest that abnormal IL-36 signalling is not a primary disease driver in this condition.

Following the identification of *IL36RN* mutations, disease associated alleles were also detected in other genes. For example, mutations in *CARD14*, a gene encoding a skin-specific NF-κB activator, has been linked to disease pathogenesis [99], [150], [151]. Despite *CARD14* mutations account for a minority of psoriatic patients, they have been found in both Ps and, more significantly, in GPP patients [152].

Whole-exome sequencing of GPP patients also revealed disease associated alleles in *AP1S3*. This encodes a protein involved in autophagosome formation which, if disrupted, leads to abnormal accumulation of p62 (an NF-kB adaptor) and up-regulation of IL-36 in keratinocytes [153]. This is an important finding, which suggests a convergence of disease pathways on the up-regulation of IL-36 signalling.

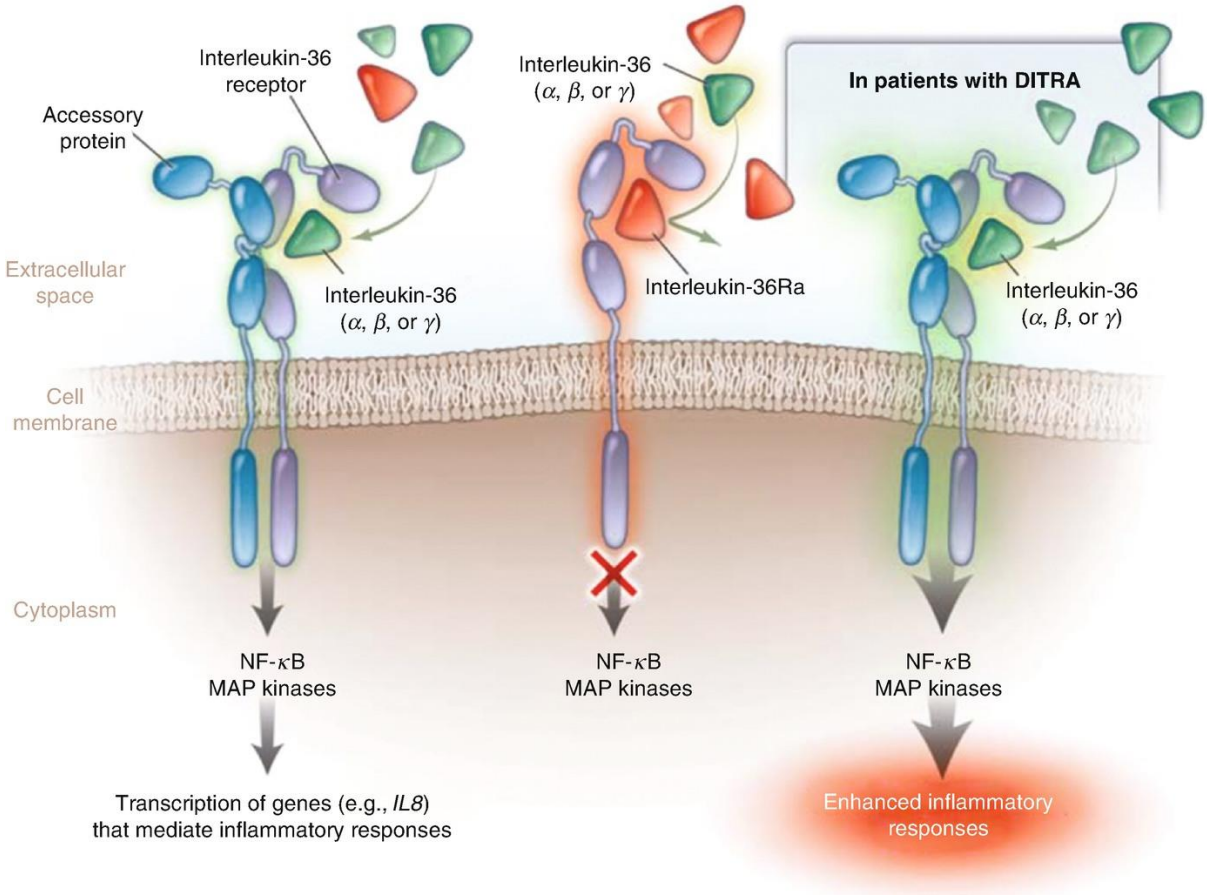


Fig. 2.3.2.2. IL-36 signal transduction.

IL-36 cytokines, similar to other IL-1 family members, can activate the MAPK and NF-κB pathways by binding to the corresponding receptor, IL-1RAcP. When IL36RN is mutated, the IL-36R antagonist cannot inhibit the binding of the other ligands leading to sustained activation of signal transduction. Adapted from [154].

2.3.2.3 Immunopathogenesis

While the immune pathogenesis of GPP was until recently very poorly understood, genetic and transcriptomic studies carried in the last few years have shed some light on disease pathways.

As the rarity of GPP has hindered the recruitment of sizeable patient cohorts, only one transcription profiling study has been undertaken in patient skin [155]. This confirmed the up-regulation of IL-1 and IL-36 cytokines, as well as the over-expression of neutrophil-derived proteases (e.g. elastase and cathepsin G), which can process IL-36 precursors into mature cytokines [156]. These changes were observed regardless of the presence of *IL36RN* mutations, confirming the notion that IL-36 plays a fundamental role in the pathogenesis of GPP.

IL-36 can up-regulate innate cytokines such as IL-1 and IL-8. While the former contributes to the acute phase response, IL-8 acts as a neutrophil chemoattractant [61], [157]. Of note, IL-36 also has an effect on endothelial cells, where it induces the expression of adhesion molecules (e.g. ICAM-1), thus facilitating the extravasation of neutrophils into inflamed skin [158].

Thus, genetic and immunological studies support the notion that GPP is an autoinflammatory condition, driven by excessive IL-36 signalling and abnormal neutrophil activation.

Despite these advances, the molecular pathways underlying the concurrence of Ps (a T-cell mediated disorder) remain ill-defined. Moreover, little is known about the role of IL-36 in the systemic symptoms of GPP.

2.3.2.4 Treatment

First line therapies for GPP include acitretin, methotrexate, cyclosporine and infliximab. While these agents are often used with good effect for the treatment of plaque psoriasis, they are often ineffective in the pustular forms of the disease [137].

Given the role of IL-1 in other autoinflammatory diseases, IL-1 receptor blockers have been used in a small number of cases, leading to rapid clinical improvements but not full disease remission [159], [160]. This supports the notion that IL-1 is not a primary disease driver, but rather a molecule that sustains inflammation, downstream of IL-36 [161].

More recently, IL-17A antagonists have shown some efficacy in GPP, however this result was obtained in small trials with no placebo arm [162], [163].

Finally, given the findings presented on this thesis and prior evidence of IL-36 role in GPP, IL-36 blockade showed to be a possible therapeutic target. Interestingly, recently published data on IL-36 blockade in GPP patients describes it as promising [164].

Such progress demonstrates the translational potential of genetic studies and underscores the need to dissect the pathways that are activated downstream of IL-36, as these may also be targeted for disease treatment.

2.4 Aims

The aim of this study was to investigate the mechanisms underlying IL-36 driven inflammation, with a particular focus on psoriasis. Thus, the intermediate objectives of the project were:

- To define a transcriptional signature of IL-36 activation, through the RNA-sequencing of IL-36 stimulated cells. This experiment was implemented in keratinocytes, based on the robust expression of IL-36R in these cells. After the presence of the signature was validated in the Ps and GPP skin transcriptomes, its biological significance was explored by means of pathway enrichment analysis.

- To investigate the pathogenic role of IL-36 signalling at the systemic level. Given the prominence of systemic symptoms and IL-36 up-regulation in GPP, the study mainly focused on this condition. The effects of IL-36 on circulating immune cells were defined through the transcription profiling of GPP leukocytes and the *ex-vivo* characterisation of IL-36 responses.

3 Materials and Methods

3.1 Materials

Reagent	Manufacturer	Catalogue number
1X KAPA SYBR FAST Universal qPCR Master Mix	Primerdesign	n/a
CD3 Isolation kit	Miltenyi Biotech	130-050-101
CFSE tracker	BioLegend	423801
CpG ODN-2216	INVIVOGEN	tlrl-2216
dNTP nucleotides	Fisher Scientific	10520651
Dulbecco's Modified Eagle Medium (DMEM), high glucose, GlutaMAX™ Supplement	Gibco	61965026
Dynabeads™ Human T-Activator CD3/CD28	Thermo Scientific	11132D
Ethanol	VWR	20821.330
Ficoll	GE	17544202
Fixation & Permeabilization kit	Thermo Scientific	GAS003
Foetal Bovine Serum (FBS)	Gibco	10500064
GeneJET RNA Purification Kit	Thermo Scientific	K0731
GLOBINclear™ Kit	Life Technologies	AM1980
Glutamax RPMI	Gibco	61870010
Human IFN-alpha ELISA kit	Bio-Techne	41100-1
IL36-α	Bio-Techne	6995-IL-010/CF
Neutrophils isolation kit	Miltenyi Biotech	130-104-434
PBS	Gibco	10010015
Penicillin-Streptomycin	Gibco	15140122
Plasmacytoid Dendritic Cell Isolation Kit	Miltenyi Biotech	130-097-415
Precision nanoScript2 Reverse Transcription kit	PrimerDesign	n/a
PrecisionPlus SYBR and ROX qPCR Mix	PrimerDesign	n/a
Recombinant Human IL-36 alpha/IL-1F6 (aa 6-158) Protein	R&D Systems	6995-IL-010
RNA buffer	Agilent	5067-5577
RNA ladder	Agilent	5067-5578
RNA screen tape	Agilent	5067-5576
RNaseZap™ RNase Decontamination Solution	Invitrogen	AM9780
RPMI 1640 Medium	Gibco	21875034
Tempus™ Spin RNA Isolation Kit	Thermo Scientific	4380204
Tryphan blue	Gibco	15250061
B2M PrimerDesign probe	PrimerDesign	54684
β-mercaptoethanol	Sigma-Aldrich	M-3148

3.2 Study resources

This research was carried out in accordance with the principles of the Declaration of Helsinki. The majority of cases (n=31) were recruited as part of the PLUM (Pustular psoriasis: eLucidating Underlying Mechanisms) study, which was approved by the London - London Bridge Research Ethics Committee (reference 16/LO/2190, 30th January 2017). A further 5 affected individuals were ascertained through the APRICOT (Anakinra for Pustular psoriasis: Response in a Controlled Trial) clinical trial, which was granted ethical approval on 1st April 2016 and assigned EudraCT number 2015-003600-23. The ethics committees of collaborating institutions also granted the required approvals. On this basis, 9 GPP patients (1 male and 8 females, average age: 57) and 7 controls (1 male and 6 females, average age 52) were ascertained for whole-blood RNA-seq. A further 35 cases (10 males, 25 females, average age: 49) and 7 controls (6 females and 1 male, average age: 45) were recruited for validation studies (Tables 3.1.1 and 3.1.2). Blood was also obtained from 4 cases (1 male and 3 females, average age: 47.3) and 4 controls (3 females, and 1 male, average age: 33.4 or 36.6) for flow-cytometry (Tables 3.2.1 and 3.2.2) and in-vitro assays. Finally, samples donated from 3 healthy volunteers (1 male and 2 females, average age: 29 or 27.6) were used for stimulation of peripheral blood mononuclear cells (PBMCs) or pDCs (see section 3.3).

All cases were diagnosed by expert dermatologists, based on the appearance of macroscopically visible sterile pustules on non-acral skin [138]. While some patients suffered from concomitant plaque psoriasis, the presence of pustules that were restricted to the edges of psoriatic plaques was considered an exclusion criterion. Details of patient demographics and clinical presentation were recorded in a standardised Case Report Form.

The patients were ascertained at St John's Institute of Dermatology (London), Glasgow Western Infirmary, Manchester University and Hospital Sultanah Aminah, Johor Bahru, Malaysia. The healthy controls were recruited from the personnel of St John's Institute of Dermatology. All study participants provided informed written consent.

Table 3.2. 1. Demographics and clinical characteristics of the affected individuals

Patient ID	Ethnicity	Sex	Age of onset	Concurrent Ps	History of systemic Inflammation[1]	Treatment	*IL36RN* status	Age at recruitment	Analysis group
T029045	European	F	10	N	Y (Fever, Neutrophilia, Elevated CRP)	U	WT	48	RNAseq
T028820	European	F	11	Y	Y (Elevated CRP)	U	p.S113L/-	47	RNAseq
T029060	European	M	5	N	Y (Neutrophilia, Elevated CRP)	No treatment	p.S113L/-	43	RNAseq
T031013	European	F	7	N	Y (Fever, Neutrophilia)	Infliximab	p.R48W/p.S113L	46	RNAseq
T026307	European	F	5	N	Y (Fever, Neutrophilia, Elevated CRP)	Methotrexate	WT	88	RNAseq
T031359	European	F	45	N	Y (Fever, Elevated CRP)	Infliximab/ Methotrexate	WT	90	RNAseq
T031360	European	F	51	N	Y (Fever, Elevated CRP)	Acitretin	p.S113L/p.S113L	65	RNAseq
T031590	Asian	F	31	Y	U	Ustekinumab/ Methotrexate	WT	47	RNAseq
T033128	European	F	29	N	Y (Neutrophilia, Elevated CRP)	Adalimumab/ Methotrexate	WT	38	RNAseq
T031846	Asian	F	24	Y	N	Acitretin	WT	44	Validation qPCR

T031852	Asian	F	40	Y	N	Acitretin	c.115+6T>C/-	65	Validation qPCR
T031858	Asian	F	17	N	N	Acitretin	WT	61	Validation qPCR
T033028	Asian	F	15	Y	N	Ustekinumab	WT	21	Validation qPCR
T033026	Asian	F	22	Y	N	Cyclosporin	WT	23	Validation qPCR
T030291	European	M	33	N	N	Methotrexate	WT	69	Validation qPCR
T031857	Asian	F	62	Y	N	Acitretin	WT	62	Validation qPCR
T033027	Asian	M	U	U	N	U	c.115+6T>C/c.115+6T>C	21	Validation qPCR
T031856	Asian	M	30	Y	N	Acitretin	U	51	Validation qPCR
T031842	Asian	M	9	N	N	Acitretin	WT	24	Validation qPCR
T031859	Asian	F	29	N	Y	Cyclosporine	WT	64	Validation qPCR

T033025	Asian	F	30	Y	N	Acitretin	WT		Validation qPCR
T031855	Asian	F	42	Y	Y	Infliximab	WT	43	Validation qPCR
T033074	Asian	F	28	Y	N	Acitretin	WT	38	Validation qPCR
T031845	Asian	F	59	N	Y	U	WT	63	Validation qPCR
T031844	Asian	F	42	Y	N	Methotrexate.	WT	42	Validation qPCR
T036368	European	F	U	U	N	Cyclosporine	WT	77	Validation qPCR
T034763	European	F	75	N	N	U	WT	74	Validation qPCR
T031848	Asian	M	73	N	Y	Acitretin	WT	73	Validation qPCR
T031854		F	U	Y	N	U	WT	U	Validation qPCR
T031121	European	F	5	N	N	Cyclosporine	WT	87	Validation qPCR

T033193	European	F	35	Y	N	U	WT	42	Validation qPCR
T039220	European	M	74	Y	N	U	WT	35	Validation qPCR
T031847	Asian	F	28	Y	Y	Adalimumab	WT	36	Validation qPCR
T031849	Asian	F	45	Y	U	Adalimumab	WT	51	Validation qPCR
T031851	Asian	F	12	Y	U	Adalimumab	c.115+6T>C/-	24	Validation qPCR
T031853	Asian	M	50	Y	U	Methotrexate	c.115+6T>C/p.P76L	U	Validation qPCR
T034763	European	F	75	N	U	U	WT	74	Validation qPCR
T036368	European	F	U	U	U	Cyclosporine	WT	57	Validation qPCR
T036888	European	M	U	U	U	Cyclosporine	WT	64	Validation qPCR
T037195	European	U	U	U	U	U	WT	42	Validation qPCR

T038523	Asian	F	30	Y	U	U	WT	29	Validation qPCR
T038546	Asian	F	41	Y	U	U	WT	U	Validation qPCR
T038988	Asian	M	Y		U	U	WT	42	Validation qPCR
T038166	U	U	U	U	U	U	U	28	Validation qPCR
T031590	Asian	F	31	N	N	Infliximab	WT	50	Flow cytometry
T036887	European	M	U	N	N	U	WT	67	Flow cytometry
T038523	Asian	F	30	Y	U	U	WT	31	Flow cytometry
T028820	European	F	42	Y	Y	Infliximab	p.S113L/-	51	Flow cytometry

[1]Fever is reported if >38C, elevated CRP= CRP>100mg/L

Y=Yes, N=No, U=Unknown, WT= wild-type

Table 3.2. 2. Demographics of the healthy individuals used as controls

Study number	Ethnicity	Sex	Age at recruitment	Analysis group
T031200	European	F	45	RNAseq
T031201	European	M	55	RNAseq
T031202	European	F	48	RNAseq
T031203	European	F	49	RNAseq
T031221	European	F	52	RNAseq
T031222	European	F	62	RNAseq
T031556	European	F	53	RNAseq
T039111	European	F	39	Validation qPCR
T039113	European	F	49	Validation qPCR
T039129	European	F	63	Validation qPCR
T039346	Asian	F	38	Validation qPCR
T039224	European	F	35	Validation qPCR
T039226	European	M	36	Validation qPCR
T031558	European	F	52	Validation qPCR
GYPLM0071	European	F	30	Cells stimulation, flow cytometry
GYPLM0037	European	M	26	Cells stimulation, flow cytometry
GYPLM0048	European	F	26	Cells stimulation
GYFAP0179	Finnish	F	31	Cells stimulation, flow cytometry
GYPLM0026	European	F	30	Flow cytometry
GYPLM0017	European	F	50	Flow cytometry
GYPLM0043	European	F	30	Flow cytometry

3.3 Transcription profiling

3.3.1 Retrieval of publicly available datasets

The Gene Expression Omnibus (GEO) and ArrayExpresss (https://www.ncbi.nlm.nih.gov/gds and https://www.ebi.ac.uk/arrayexpress/) databases were queried using the “tissue”, ”date” and “experiment” search fields, in order to identify transcriptome studies (RNAseq, microarray) performed on whole blood, no later than December 2015. This search uncovered four datasets including individuals affected by cryopyrin-associated periodic syndrome (CAPS), interferonopathies or plaque psoriasis (Table 3.3.1). Raw counts or fastq files were downloaded and analysed using the in-house pipeline described in the following sections.

The 10KImmunome [165] dataset (whole blood transcriptome profiles for 221 healthy individuals. 110 males and 111 females, average age: 31, ethnicity: 44 Asian, 126 White, 25 Black and African American, 26 Others) was downloaded as normalised counts (Table 3.3.1).

Table 3.3. 1. Study resources

Dataset ID	Dataset	Cohort	Reference
GSE55201, GEO DataSets	Ps, whole blood	33 cases, 44 healthy controls	Wang CQF, et al. J Invest Dermatol 2014 [166, p. 17]
n/a; data provided by the authors	CANDLE, whole blood	3 cases, 5 healthy controls	Brehm A., et al. JCI 2015 [19]
E-MTAB-5735	type I interferon-mediated autoinflammation, whole blood	8 cases, 5 healthy controls	Rodero MP., et al. Nat Commun 2017 [167]
GSE57253, GEO DataSets	NOMID, whole blood	7 cases, 11 healthy controls	Canna SW., et al. Nat Genet 2014 [168]
GSE67785, GEO DataSets	Ps, skin	14 paired lesional and non lesional skin biopsies	Swindell, W. R., et al. Genome Med 2015 [169]
GSE59275, GEO DataSets	IL-4 treated keratinocytes	3 replicates of treated and untreated keratinocytes	Only available through GEO Datasets
n/a; data downloaded from: http://10kimmunomes.ucsf.edu/	Human whole blood transcriptome	221 healthy individuals	Zalocusky K.A., et al. Cell Rep 2018 [165]

3.3.2 RNA isolation and sequencing

Three ml of whole blood were obtained from each study participant and stored at -80C in a Tempus™ Blood RNA Tube. Total RNA was isolated using a Tempus™ Spin RNA Isolation Kit, according to the manufacturer's instructions. Samples were then diluted in a final volume of 50 µl of RNAse free water.

Samples were subject to globin depletion using a GLOBINclear™ Kit and quantified with a Qubit 4 fluorometer (Thermo Fisher Scientific). RNA integrity numbers (RIN) were measured with a Tapestation 4200 (Agilent Genomics). RIN were higher than 7 in all samples.

RNA-seq libraries were prepared with the Illumina TruSeq Stranded polyA RNA preparation kit and sequenced on an Illumina HiSeq 3000 system at the Next Generation Sequencing Facility, Leeds Institute of Molecular Medicine, University of Leeds. Data were provided as fastq files and analysed as described below.

3.3.3 Differential expression analysis

3.3.3.1 RNAseq analysis

The quality of the sequence data was assessed using FastQC and reads with a Phred score<30 were excluded from further analyses. The reads that passed this step were trimmed and overrepresented sequences were removed using FASTX-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/). The alignment was performed against the HG38 human genome using TopHat [170], with indexes generated by Botwie2 and reports by samtools [171]. Finally, the reads aligning to each gene were counted, using HTseq-count (--nonunique union, --idattr=gene_id).

Read counts were used as input for the differential expression analysis. This was performed using DESeq2 (R package, v 16.2, [172]). The underlying methodology may be summarised as follows: read counts for each gene are described with a generalised linear model (GLM) of the negative binomial family. The model uses the mean read counts per gene per sample, normalised with a sample-specific constant that takes into account the total number of reads obtained in each sample. The mean normalised gene counts are used to estimate gene dispersion and compute a trend. Gene specific dispersion is then fitted to the trend using the GLM model and taking into account within-group variability. At this stage, confounding factors can also be added to the equation as covariates.

Fold changes are computed postulating a zero-centred normal prior, meaning that the distance of each gene from the fitted trend is the measure of the differential expression of the contrast group. Finally, the P values are calculated using the Wald Test and then corrected for multiple testing using Benjamini–Hochberg procedure [173].

3.3.3.2 Microarray analysis

The analysis of microarray data was undertaken with the limma package [174], which is based on a linear model. A correction is first performed to subtract the background noise from the foreground intensity of each spot. Signals are then normalised using Robust Multichip Average (RMA) normalization methods, and converted into expression measures. Two matrices are built: the design (to specify contrast groups) and the coefficient matrix. Each row of the design matrix corresponds to an array in the experiment and each column corresponds to a coefficient that is used to describe the RNA sources. A linear model is fitted for every gene and a Bayes method is used to assess differential expression [175].

3.3.4 Enrichment analyses

3.3.4.1 Pathway enrichment analysis

After differential expression analysis, genes with an absolute fold change (FC) ≥1.5 and a false discovery rate (FDR) <0.05 were used as input for Ingenuity Pathway Analysis (IPA, Qiagen). Briefly, the software first ranks the genes based on FC and then assigns a weight to each feature. Input genes are then mapped against reference pathways and an enrichment P value is computed with an exact Fisher's test, taking into account the size of the pathway and the weight of differentially expressed genes. P values are then corrected for multiple testing using the Benjamini-Hochberg method.

3.3.4.2 Transcription Factor Activity

TF activity analyses was also performed using IPA software, using the *Upstream Analysis* option. The algorithm considers the dataset as a subgraph with a priori unknown causal edges in the master network. In order to identify them, all the genes are considered as potential regulators and they are scored for downstream targets identified. The downstream targets are defined by an enrichment P value assuming a random dataset with a constant number of genes as null model. To each network is then associated an activation score based on co-expression values. Finally, the enrichment of each network in the dataset is computed [176].

3.3.4.3 Transcriptional Module enrichment

The script published by Li et al. [177] was slightly modified, in order to identify blood transcriptional modules that are enriched in the GPP transcriptome. Briefly, the modules that were active in our dataset were selected using the genetable_to_activityscores function. Next, the enrichment_test function was applied to our list of differentially expressed genes, taking into account the module activity scores and fold change of the genes mapping to each module. Enrichment P values were then calculated and corrected for multiple testing, applying a significance threshold of FDR <0.05.

3.3.4.4 Cytokine scores

The interferon score has been previously defined and used by others [178] as an indirect measure of type-I-IFN production. Here, five interferon stimulated genes (ISGs: *IFI6, IFIT3, IFITM3, OASL* and *PLSCR1*) were identified by cross-referencing the most up-regulated loci in GPP blood with the type I IFN targets reported in the Interferome database [179]. Since the genes that are induced by IL-36 have not been characterised in leukocytes, the IL-36 signature was defined by using genes that were i) up-regulated in KCs stimulated with IL-36 [180] and ii) expressed in blood (*IL1B, PI3, VNN2, TNFAIP6* and *SERPINB1)*. For both measures, the expression of the five signature genes was normalised against a calibrator and scores were computed in each individual, as the median normalised expression of the five genes.

3.4 Cell culture and stimulation

3.4.1 Cell isolation

Blood (20 ml) was collected from healthy volunteers and peripheral blood mononuclear cells (PBMCs) were isolated by density centrifugation on 15 ml of Ficoll, in the absence of red blood cells lysis. Cells were counted on a Marenfield superior haemocytometer and seeded in 48-well plates, at a density of $2.5x10^6$cells per well.

Plasmacytoid dendritic cells (pDCs) were purified from PBMCs using a Plasmacytoid Dendritic Cell Isolation Kit (Miltenyi Biotec) and cultured at a density of $2.5x10^5$ cells/ml.

T cells were isolated using a CD3 Isolation kit (Miltenyi Biotec) and cultured at a density of $1.5x10^6$ cells/ml. Cells were labelled with CFSE tracker (BioLegend) at time zero and stimulated with Dynabeads™ Human T-Activator CD3/CD28 for up to 72 hours. Cells were then stained for FACS analysis.

3.4.1 Cell culture

All cells were grown under aseptic conditions in a NUAire Air-Jacketed Automatic CO_2 (NU-5500) incubator at 37C and 5% CO_2.

Freshly isolated cells were incubated overnight in Glutamax RPMI supplemented with 10% FBS and 5% P/S (Penicillin/Streptomycin Solution). The next morning the cells were stimulated with 50ng/ml IL36-α for 6 hours and then with 1.6µg/ml of ODN-A CpG (or vehicle) for a further 6 or 12 hours.

Cells were then harvested and used for FACS assays or centrifuged at 3000 rpm for 7 minutes. Supernatants and cell pellets were stored at -80C for use in ELISA or RNA extraction, respectively.

Each experiment was performed at least three times, in three technical replicates.

3.4.2 Quantitative real-time PCR

Prior to RNA extraction, all surfaces and pipettes were treated with RNaseZap RNase Decontamination Solution. RNA was extracted from whole blood using Tempus™ Spin RNA Isolation Kit and from PBMCs using the GeneJET RNA purification kit. RNA was eluted in 50 and 30 µl of RNase-free water, respectively. Samples were quantified using Qubit 4 fluorometer (Invitrogen) and stored at -80°C.

For each experiment, 100-200ng of RNA were reverse-transcribed with a nanoScript2 kit. The reactions were incubated at 25°C for 10 minutes, 37°C for 120 minutes and 85°C for 5 minutes. After the reverse transcription was completed, the cDNAs were diluted by adding 30 µl of nuclease-free water to each reaction.

Gene expression was assessed using the primers listed in Table 3.4.1. Primers were designed using Primer3 web version 4.1.0 [181]. Reactions were set up to a final volume of 20µl containing 70nM of primers, 1X KAPA SYBR FAST Universal qPCR Master Mix and 2µl of cDNA.

Samples were loaded on a 7900HT Fast Real Time PCR System (Applied Biosystem) and the cycling conditions were set up as follows: 95° for 10 minutes, (95° for 10 seconds, 60° for 60 seconds) x 40.

Transcript levels were normalised using *B2M* expression and relative gene expression was quantified using the ΔΔCt method [182].

Table 3.4.1. Primers used for the real time PCR

Target gene	Primer name	Sequence
IFI6	IFI6-R	TTTCTTACCTGCCTCCACCC
	IFI6-F	CCATCTATCAGCAGGCTCCG
IFIT3	IFIT3-R	TTGGTGACCTCACTCATGATGG
	IFIT3-F	GCACAGACCTAACAGCACCC
IFITM3	IFITM3-R	CACTGGGATGACGATGAGCA
	IFITM3-F	TCGCCTACTCCGTGAAGTCTA
OASL	OASL-R	GGAACCTGGAAGGACAGACG
	OASL-F	GTACCAGCAGAGGGCACG
PLSCR1	PLSCR1-R	AGGAGGATACCCAACTGGCA
	PLSCR1-F	CGGCAGCCAGAGAACTGTTTTA

All the primers were provided by SIGMA ALDRICH.

3.4.3 ELISA

The production of IFN-α was measured with the Human IFN-α ELISA kit. Supernatants were diluted 1:10 and analysed in duplicated. The absorbance at 450nm was determined using an ELISA FLUOstar Omega reader (BMG Labtech). Raw data was analysed using a 4-parameter fit with an in-house R script.

3.5 Flow cytometry

PBMCs and T cells were isolated from 20ml blood samples obtained from four healthy controls. Neutrophils were purified from four ml blood samples (n = 3 healthy volunteers), using the MACSxpress Neutrophil isolation kit. pDCs were purified from freshly isolated PBMCs (n = 3 healthy volunteers). pDCs, PBMCs and neutrophils were seeded in five ml round-bottom polystyrene tubes at a density of 10^5 cells/100µl , 2.5x10^6cells /100µl and 1x10^6cells /100 µl, respectively. Cells were washed with two ml PBS before centrifugation at 1800 rpm for 5 minutes.

For live cell detection, LIVE/DEAD™ Fixable Near-IR was added at a 10nM final concentration. Cells were then incubated with the dye at 4° for 15 minutes. After a PBS wash Monocytes and Fc blocker (1:40) was added for 20 minutes. PBMCs were washed and treated with two different combination (panels) of antibodies (Table 3.5.1), in order to identify the various leukocyte populations that were present in the sample. All incubations were performed in the dark. Neutrophils were stained for Lineage cocktail, CD15, CD16 and CD14. T cells were stained for CD3, CD4 and CD8.

For IFN-α and PLSCR1 detection, cells were fixed using a Fixation & Permeabilization kit prior to intracellular staining. In all experiments, cells were incubated for 20 minutes at 4° with the relevant antibody and then washed in PBS (for surface staining) or Perm buffer (for intracellular straining). When needed, the secondary antibody was added for 20 minutes at 4°; cells were then washed and re-suspended in 300µl of PBS or Perm buffer, as appropriate.

Cells were analysed on a BD Fortessa LSR machine, collecting 10^5 events. Data were analysed using FlowJo v10 software. The gating strategy is summarised in Fig. 3.5.1.

Table 3.5.1. Flow Cytometry Antibodies

Antibody	Cat Number	Colour	Provider	Diluition[1]	Application
CD16	48-0168-42	Efluor450	ThermoFisher	1:20	Panel 1 / Neutrophils
CD56	318316	Alexa Fluor 700	BioLegend	1:33	Panel 1
CD19	302242	BV510	BioLegend	1:20	Panel 1
CD20	130-096-649	PE-Cy7	Miltenyi	1:33	Panel 1
CD14	555398	PE	BD	1:20	Panel 1 / Neutrophils
CD3	317306	FITC	BioLegend	1:33	Panel 1/T cells
CD127	351320	PE-Cy7	BioLegend	1:20	Panel 2
HLA-DR	307636	BV421	BioLegend	1:33	Panel 2/pDCs
CD11c	301638	BV650	BioLegend	1:20	Panel 2/pDCs
CD123	306030	BV711	BioLegend	1:30	Panel 2/pDCs
Lineage Cocktail [2]	B29559	PE	Beckman Coulter	1:10	Panel 2
CD15	301904	FITC	BioLegend	1:33	Neutrophils
IL1RL2	BAF	Streptavidin	BD	1:10	Panel 1/ 2 and Neutrophils
Biotinylated secondary Antibody[3]	405207	APC	BioLegend	1:100	Panel 1/ 2 and Neutrophils

PLSCR1	ab180518	Rabbit-IgG	Abcam	1:50	Panel 1/ 2 and pDCs
Rabbit IgG[4]	406416	Alexa Fluor 488	BioLegend	1:100	Panel 1/ 2 and pDCs
IFN-α	130-092-602	APC	Miltenyi Biotec	1:10	Panel 1/ 2 and pDCs
CD4	357424	BV421	BioLegend	1:33	T cells
CD8	555750	PE-Cy5	BioLegend	1:33	T cells

[1]Final volume = 100 µl

[2]CD3/CD14/CD19/CD20/CD56; [3]Target of secondary biotinylated antibody used for IL36R detection; [4]Target of secondary antibody used for PLSCR1 detection

Fig. 3.5.1. Gating Strategy.

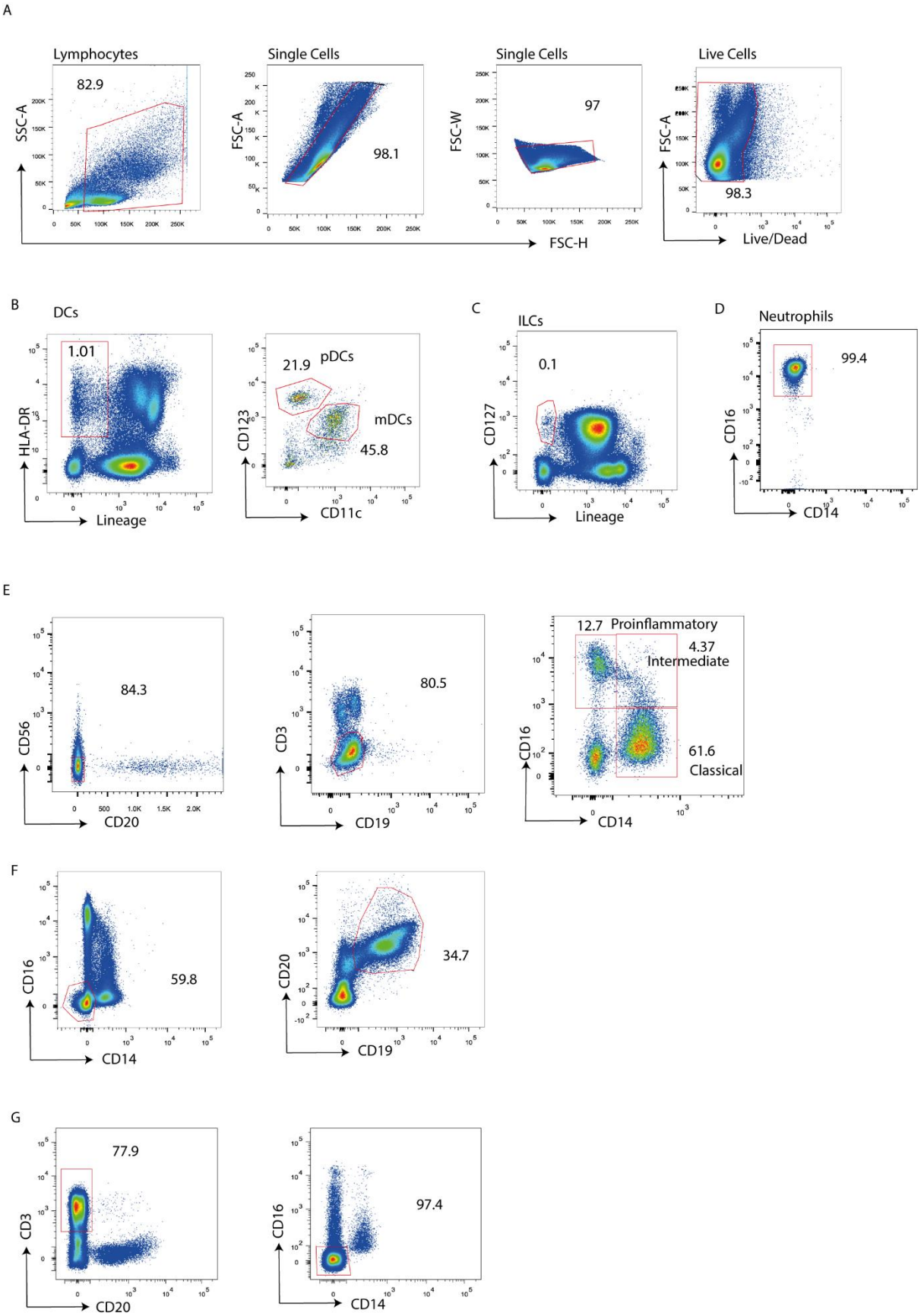


Fig 3.5.1 Gating Strategy

(**A**) General gating strategy applied to all the samples in which debris, doublets and dead cells are removed. (**B**) Dendritic cells (DCs) are gated as HLA-DR^{+} Lineage^{-} cells and then separated into pDCs and myeloid dendritic cells (mDCs), based on CD123 and CD11c expression. (**C**) Innate lymphoid cells (ILCs) are gated as CD127^{+} Lineage^{-} cells. (**D**) Neutrophils are gated as CD16^{+} CD14^{-} CD15^{+} cells. (**E**) Monocytes are selected as CD56^{-}, CD20^{-}, CD3^{-}, CD19^{-} cells and then separated into pro-inflammatory (CD16^{high} CD14^{-}), intermediate (CD16^{+} CD14^{+}) and classical (CD16^{-} CD14^{+}) subtypes. (**F**) B cells are selected as CD14^{-} and CD16^{-} cells, and successively gated for CD19 and CD20. (**G**)T cells are CD3^{+}, CD20, CD14^{-} and CD16^{-}.

4 IL-36 cytokines have a key role in the pathogenesis of psoriasis

4.1 IL-36α, -β and -γ induce similar signature genes in keratinocytes

To achieve a better understanding of IL-36 activity in psoriasis, IL-36 signature genes were defined by expression profiling of primary keratinocytes (KCs).

KCs isolated from healthy donors were treated with IL-36α, -β or -γ for 24h. RNA was then isolated from technical replicates (n=3 for each condition) and sequenced. On average 24.5 million reads were mapped in each sample, yielding a mapping rate of 95.15% (Table 4.1.1).

Table 4.1.1. RNAseq coverage statistics

				Mean read count per gene	
Sample description		*Mapped paired-end reads*	*Mapping rate*	*Raw count*	*Normalised count*
IL-36α treated KCs	replicate-1	23,920,393.00	95.00%	612.44	639.59
	replicate-2	24,318,945.00	95.30%	612.50	632.08
	replicate-3	25,239,033.00	95.10%	646.79	631.58
IL-36β treated KCs	replicate-1	23,068,715.00	95.10%	591.21	609.28
	replicate-2	24,188,385.00	95.00%	616.96	619.65
	replicate-3	21,578,713.00	95.00%	552.08	622.24
IL-36γ treated KCs	replicate-1	26,137,519.00	95.00%	670.96	660.01
	replicate-2	25,336,550.00	95.50%	650.79	661.20
	replicate-3	26,381,790.00	95.20%	669.91	636.71
untreated KCs	replicate-1	26,001,336.00	95.20%	663.49	614.60
	replicate-2	25,479,797.00	95.20%	657.08	626.87
	replicate-3	23,255,657.00	95.20%	592.66	630.45

Principal component analysis (PCA) of normalised read counts showed a very clear separation between untreated and treated KCs. Conversely, the three groups of stimulated KCs clustered very close to each other (Fig. 4.1.1 A). This pattern was confirmed by hierarchical clustering analysis (Fig.4.1.1 B), suggesting significant similarities between the genes induced by IL-36α, β and γ.

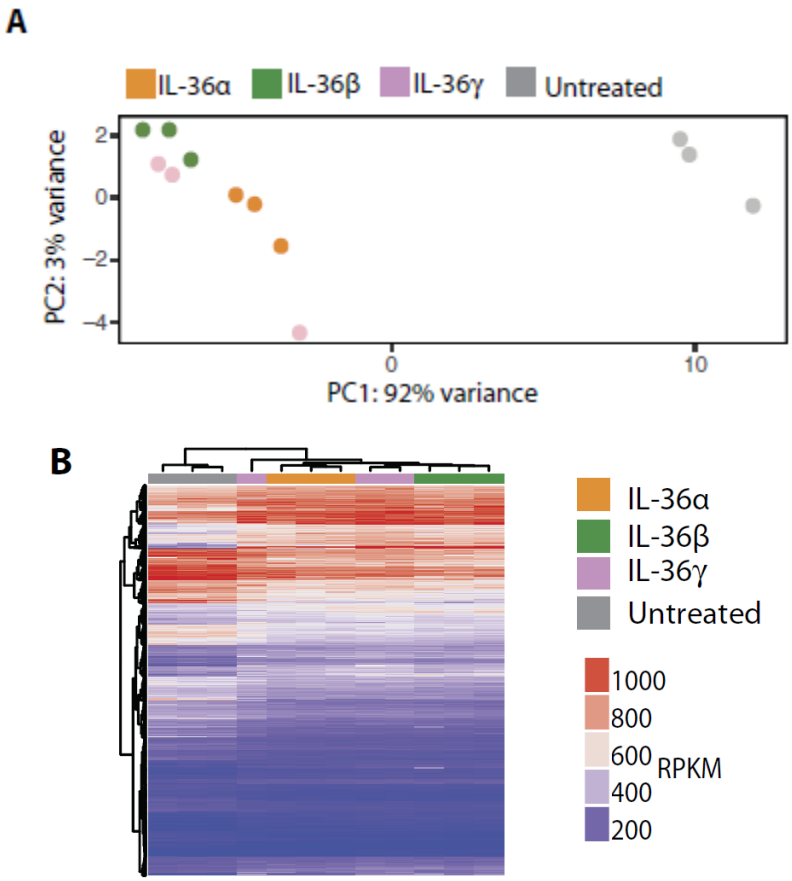


Fig. 4.1.1. Features of IL-36α, -β and -γ transcriptomes in keratinocytes.

(A) PCA showing that untreated keratinocytes cluster separately from the treated ones. Cultures were stimulated in triplicate, so each dot represents a technical replicate. (**B**) Heatmap generated using unsupervised clustering algorithm from normalised expression values (RPKM) showing differences and similarities between the samples.

A comparison of treated and untreated samples demonstrated that 4,096, 4,459, and 3,468 genes were differentially expressed [false discovery rate (FDR), <0.05] upon treatment with IL-36α, IL-36β, and IL-36γ, respectively (Fig.4.1.2). Of these, 207, 352, and 229 were up-regulated by at least twofold.

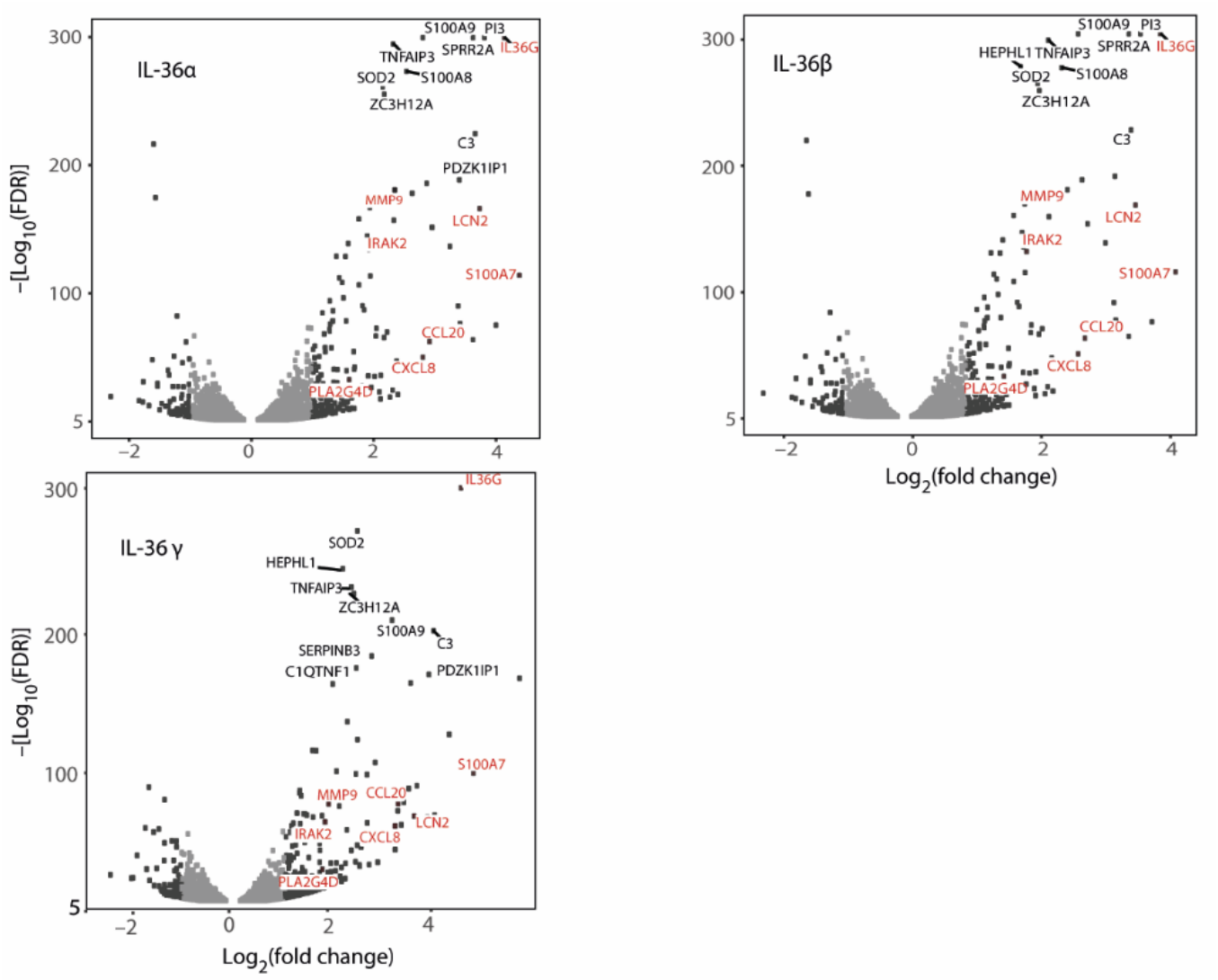


Fig. 4.1.2. Differentially expressed genes in keratinocytes treated with IL-36 cytokines.

Volcano plot showing differentially expressed genes in KCs treated with IL-36α,β or ɣ (from top left, respectively). Black dots represent genes differentially expressed at FDR < 5% and absolute $\log_2$ FC ≥ 1.5. Names for the most up-regulated genes are reported. Red labels indicate the genes validated by real time quantitative PCR (see 4.5).

Only a small number of genes were specifically up-regulated by IL-36α or γ (n=3 and 5, respectively). Conversely, 112 of the 352 genes (31.8%) that were induced by IL-36β were only up-regulated by this cytokine. These unique targets included several long non-coding and antisense RNAs, suggesting that IL-36β may drive important regulatory circuits in skin.

Overall, a substantial overlap between the three datasets was observed, as 182 genes (referred to as the IL36_182 set from here on) were induced by all three cytokines (Fig 4.1.3).

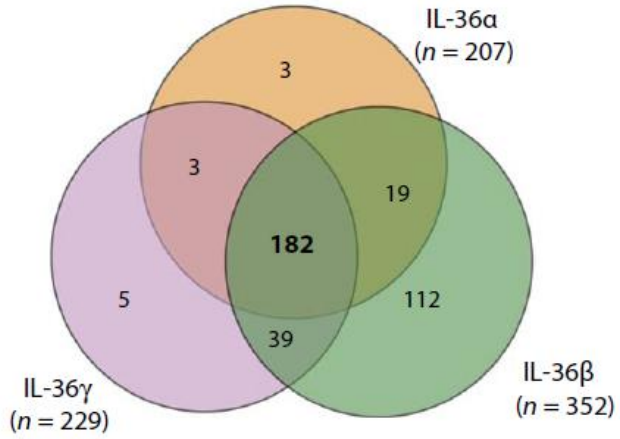


Fig. 4.1.3. Identification of IL36_182 gene set.

Venn diagram showing the overlap between genes that are up-regulated by IL-36α (orange), β (green) or γ (purple). The numbers of up-regulated genes for each dataset are reported in brackets.

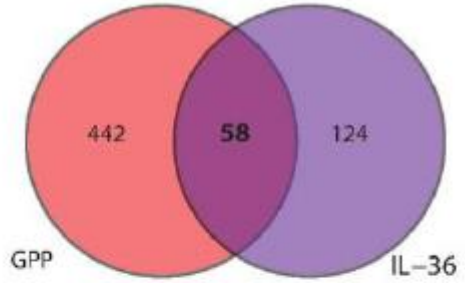
The IL36_182 set includes genes that were previously known to be IL-36 regulated (*CCL20, CXCL1, IL8, DEFB4A, STEAP4*) [183], as well as novel targets such as the phospholipase *PLA2G4D*, which is involved in lipid antigen presentation by Langerhans cells [27]. Furthermore, an interesting up-regulated gene shared by the three dataset is *OASL*. This gene is involved in antiviral response and production of type-I IFN. In fact, this cytokine plays a key role in inflammation and in psoriasis as previously described [74] and will be further investigated in the next chapters.

4.2 IL-36 is a dominant cytokine in the GPP and Ps skin transcriptomes

Having identified a set of IL-36 signature genes, the next step was to establish whether these were enriched within the GPP and Ps transcriptomes. Two publicly available datasets were examined: a GPP microarray resource (based on the analysis of skin biopsies obtained from 30 cases and 20 healthy controls) and a Ps RNAseq study (based on the analysis of 44 Ps and 30 control skin samples) [169], [184].

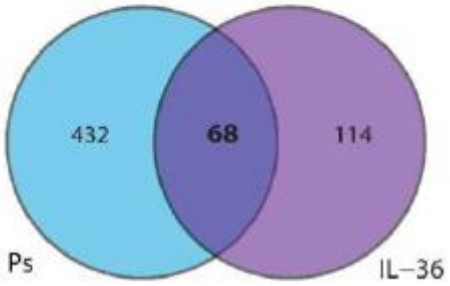
As expected for an IL-36 driven disease, the analysis of the GPP resource revealed a significant overlap with the IL36_182 set ($P<10^{-15}$), as 58 genes were up-regulated by IL-36 and robustly over-expressed in patient skin (Fig. 4.2.1). Of note, a similar enrichment was observed in the Ps dataset, with 68 of the IL36_182 loci found to be highly over-expressed in patient skin ($P<10^{-15}$) (Fig. 4.2.1).

To further validate these results, the transcriptome of KCs treated with IL-4 (an irrelevant Th2 cytokine) was analysed as a negative control. As expected, the gene set induced by IL-4 did not significantly overlap with the GPP or Ps transcriptome ($P>0.03$, Fig. 4.2.2).



Top IL-36 upregulated genes in GPP transcriptome

DEFB4A	*RHCG*
IL36G	*S100A7*
LCN2	*S100A7A*
PI3	*SERPINB4*
PDZK1P1	*SPPR2E*



Top IL-36 upregulated genes in Ps transcriptome

DEFB4A	*S100A7*
IL36G	*S100A7A*
LCN2	*SERPINB4*
PI3	*SPPR2A*
RHCG	*VNN3*

Fig. 4.2.1.The IL36_182 set significantly overlaps with genes up-regulated in GPP or Ps.

Venn diagrams showing the overlap between the IL36_182 set (purple) and the 500 genes that are most significantly up-regulated genes in GPP (red) or Ps (blue). The 10 IL-36_182 genes that show the most significant over-expression in the Ps or GPP datasets are listed beside each diagram.

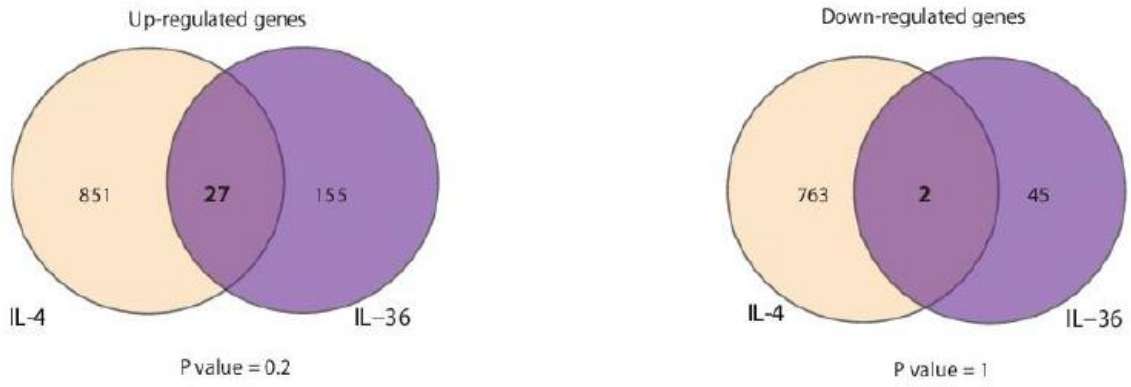


Fig. 4.2.2. IL-4 signature genes do not overlap with the Ps or GPP transcriptome

Intersection between the IL36_182 set and genes that are up- (FDR < 0.05, log2 Fold Change > 2) or down- (FDR < 0.05, log2 Fold Change < -2) regulated by IL-4 in keratinocytes. The P values under each diagram show that the overlap is not significant.

4.3 IL-36 induced genes are enriched within Ps susceptibility loci

To explore the possibility that IL-36 induced genes may be genetically linked to psoriasis, their overlap with psoriasis susceptibility loci previously identified by Genome Wide Association Studies (GWAS) was examined.

Interestingly, this analysis demonstrated that the IL36_182 set was significantly enriched among the genes lying within psoriasis associated regions ($P=9.1x10^{-4}$). To further support these findings, genes associated with schizophrenia or autism were investigated as negative controls. As expected, no significant overlap was observed between IL-36 induced genes and the susceptibility loci for the above diseases ($P>0.05$) (Fig.4.3.1).

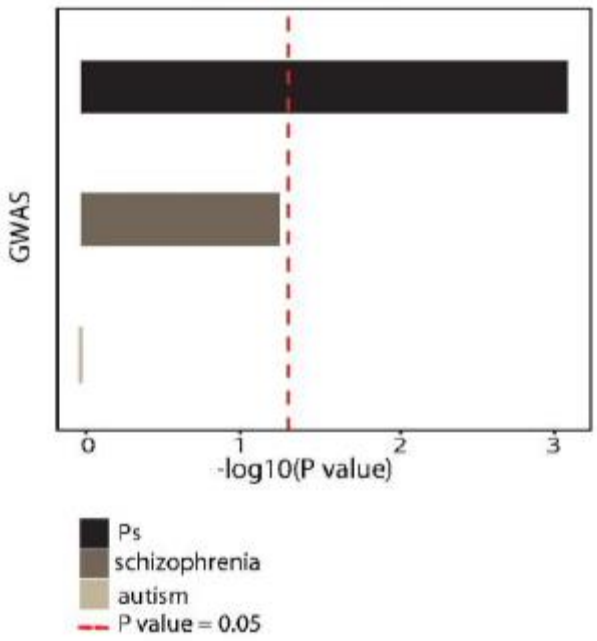


Fig. 4.3.1. Members of the IL36_182 set are over-represented within psoriasis susceptibility intervals.

Bar chart showing the over-representation of the IL36_182 set among genes lying within Ps susceptibility regions detected in GWAS. Schizophrenia and autism susceptibility intervals were analyzed as negative controls.

4.4 IL-36 signature genes cluster to pathways implicated in the pathogenesis of psoriasis

The following phase of this study aimed to link the IL36_182 genes to their biological function. To this end, pathways analyses were performed using IPA. This identified 58, 54 and 63 enriched pathways (FDR < 0.05) in KCs stimulated with IL-36α, -β or -γ, respectively.

Interestingly, the number of pathways that were associated with a single cytokine was very low (3, 3 and 2 for IL-36α, -β and –γ, respectively), with the majority (45/63) showing an enrichment in all three transcriptomes (Fig 4.4.1) (Appendix A).

The most substantially enriched pathway was *Role of IL–17A in Psoriasis* (FDR $<10^{-10}$in all three datasets) with significant FDR values also observed for several processes related to inflammation. These included *Acute Phase Response Signalling* (FDR $<10^{-5}$), *Communication between Immune Cells* (FDR $<10^{-5}$), *Toll-like Receptor Signalling* (FDR $<10^{-5}$) and *Granuocyte/Agranulocyte Adhesion in DIapedesis* (FDR $<10^{-8}$) (Fig 4.4.2).

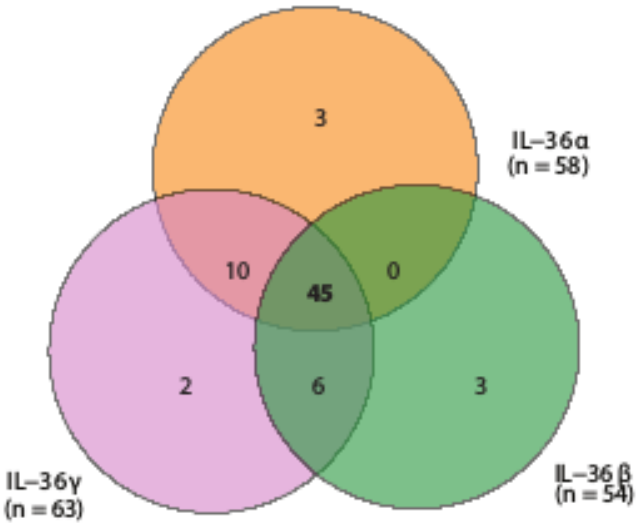


Fig. 4.4.1. There is a substantial overlap between the pathways that are enriched in the IL-36α, -β and -γ transcriptomes.

Venn diagram showing the intersection of the pathways that are enriched (FDR<0.05) in the three IL-36 transcriptomes. The number of enriched pathways for each dataset is reported in parentheses.

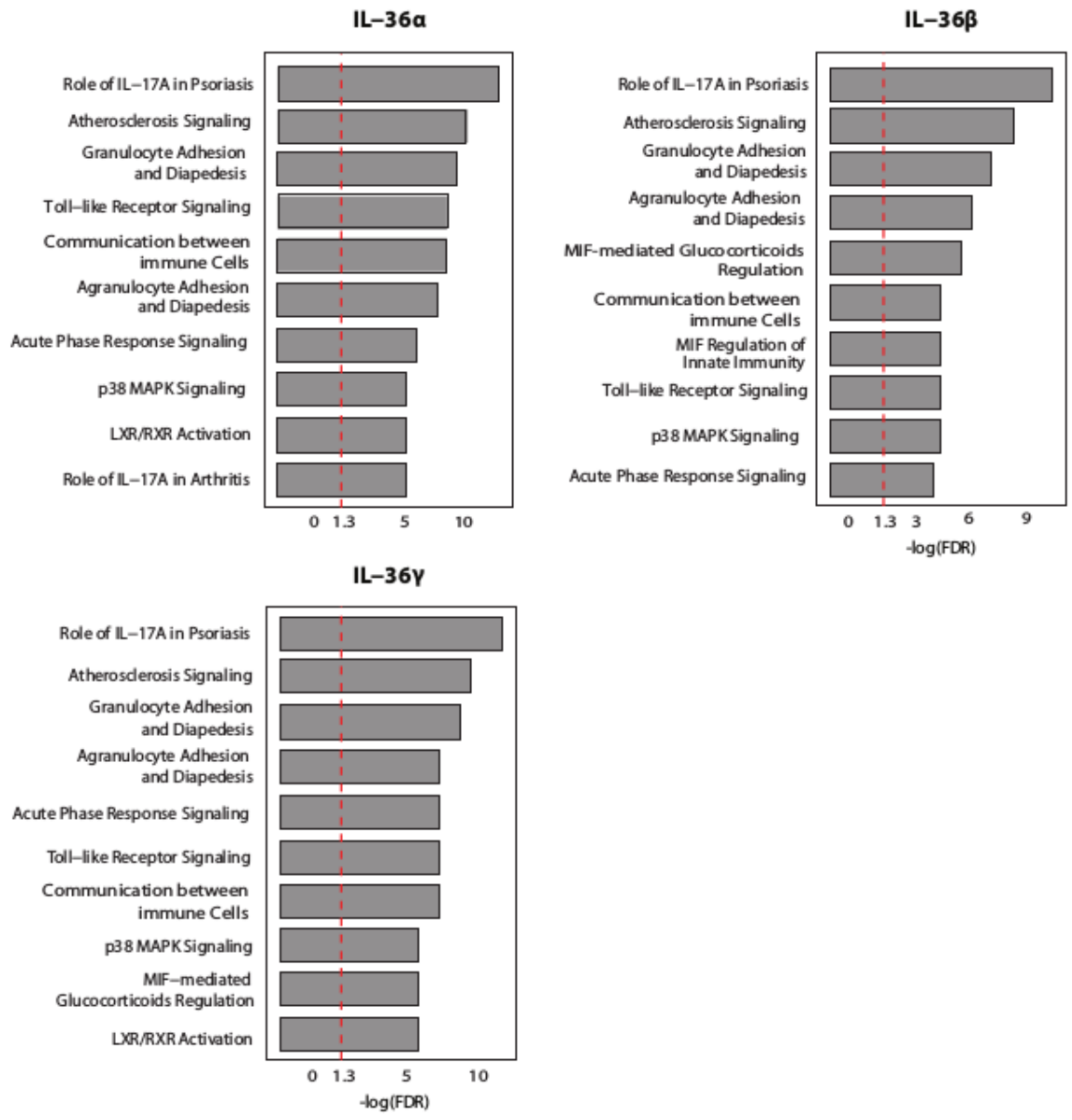


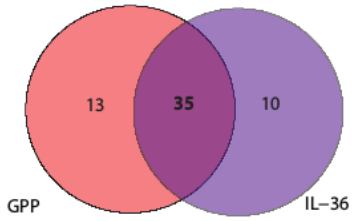
Fig. 4.4.2. The biological pathways driven by IL-36α, -β and -γ are relevant to the pathogenesis of psoriasis.

Pathway enrichment analysis demonstrates that processes related to psoriasis, e.g. IL-17 signaling and leukocyte recruitment, are over-represented in the IL-36α, -β and -ɣ transcriptomes. The red dotted line shows the –log(FDR) level corresponding to an FDR<0.05.

To further investigate these findings, a pathway analysis was also performed for the genes that are up-regulated in Ps and GPP. This uncovered 71 enriched pathways in Ps and 48 in GPP. Of note, both sets showed a significant overlap ($P<10^{-10}$) with the 45 pathways driven by IL-36 cytokines (Fig. 4.4.3).

Most significantly enriched pathways shared between IL-36 and Ps

Canonical Pathway	-log(FDR)			
	IL–36α	IL–36β	IL–36γ	Ps
Role of IL–17A in Psoriasis	13.1	11.0	12.6	11.0
Atherosclerosis Signaling	9.8	9.6	10.2	11.0
Granulocyte Adhesion and Diapedesis	7.8	7.3	9.2	19.9
Agranulocyte Adhesion and Diapedesis	6.8	6.2	6.9	13.2
Communication between immune Cells	7.0	5.1	6.7	9.1
LXR/RXR Activation	5.2	4.0	5.8	5.4
Toll–like Receptor Signaling	7.0	5.1	6.7	3.4
Differential Regulation of Cytokines Production in Intestinal Cells by IL-17A and IL-17F	4.5	3.4	4.3	8.4
TREM1 Signaling	4.1	3.4	3.8	3.9
Role of IL-17 in Arthritis	5.2	3.7	5.0	3.3



Most significantly enriched pathways shared between IL-36 and GPP

Canonical Pathway	-log(FDR)			
	IL–36α	IL–36β	IL–36γ	GPP
Atherosclerosis Signaling	9.8	9.6	10.2	13.2
Role of IL–17A in Psoriasis	13.1	11.0	12.6	7.3
Granulocyte Adhesion and Diapedesis	7.8	7.3	9.2	5.9
Agranulocyte Adhesion and Diapedesis	6.8	6.2	6.9	4.8
Acute Phase Response Signaling	5.5	4.8	6.7	6.1
p38 MAPK Signaling	5.2	5.0	5.9	4.8
LXR/RXR Activation	5.2	4.0	5.8	5.6
Communication between immune Cells	7.0	5.1	6.7	3.9
Differential Regulation of Cytokines Production in Intestinal Cells by IL-17A and IL-17F	4.5	3.4	4.3	4.5
Role of IL-17F in Allergic Inflammatory Airway Diseases	4.2	3.1	5.1	3.4

Fig. 4.4.3. IL-36 induced genes map to pathways related to psoriasis.

The Venn diagram shows the intersection between the pathways that are enriched in Ps (blue) or GPP (red) and in the three IL-36 transcriptomes (purple). The 10 most significantly enriched pathways are reported beside each diagram.

4.5 Validation of selected IL-36 target genes

To validate the effect of IL-36 cytokines on downstream genes, keratinocytes were obtained from three additional healthy donors. Following stimulation with IL-36α, -β or -γ, the expression of genes mapping to pathways enriched in Ps skin (highlighted in red in Fig 4.1.2) was examined. The analysis specifically focused on key loci related to *Role of IL-17A signalling in psoriasis* (*IL36G*, *S100A7* and *LCN2*); *Granulocyte/agranulocyte adhesion and diapedesis* (*CCL20*, *IL8* and *MMP9*) and *p38 MAPK signalling* (*IRAK2* and *PLA2G4D*).

Real-time PCR confirmed that IL-36 cytokines up-regulated all examined genes. The effect was particularly pronounced for *S100A7* (FC >50) and *IL36G* (FC > 8.5) (Fig 4.5.1). Thus, the up-regulation of psoriasis-related genes by IL-36 cytokines was readily validated through the analysis of independent donors.

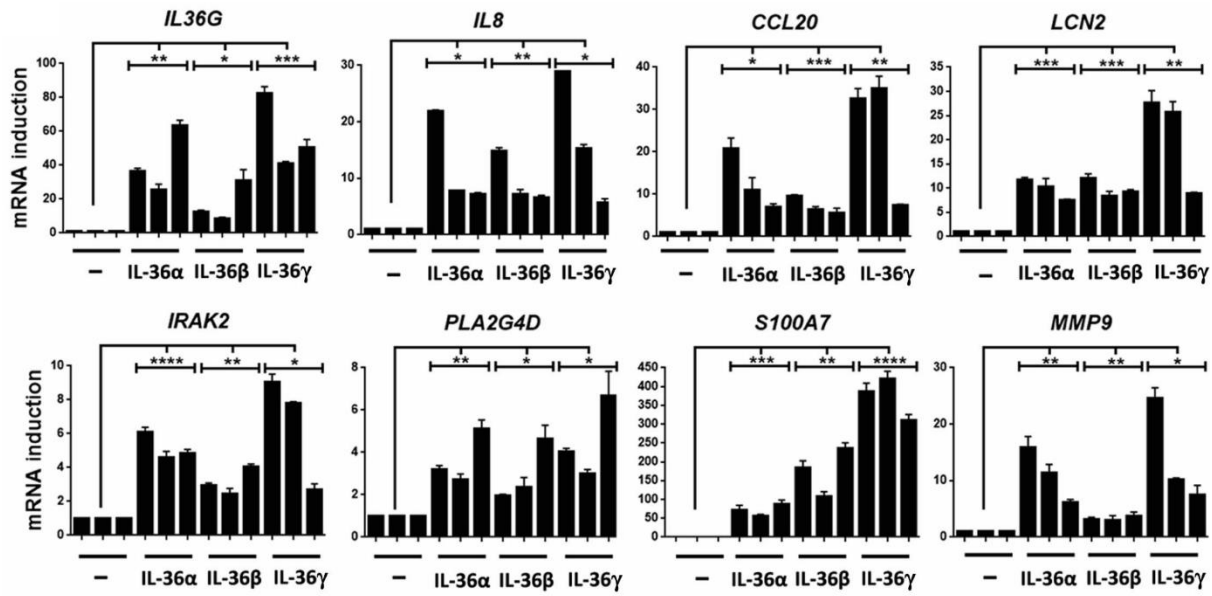


Fig. 4.5.1. Validation of up-regulated genes in keratinocytes obtained from independent healthy donors.

mRNA induction of key genes was measured by real-time PCR, using *B2M* as an endogenous control. Within each experiment, gene expression measurements were normalised to the transcript levels observed in the untreated sample (-). Data are presented as mean +/- SD of technical duplicates. Each bar represents one of the three independent donors. *P < 0.05, **P < 0.01, ***P ≤ 0.001, ****P ≤ 0.0001 (Two-way ANOVA with Dunnet's post-hoc test).

4.6 Discussion

The aim of these experiments was to investigate the role of IL-36 cytokines in the pathogenesis of psoriatic skin inflammation.

Several transcriptomic studies have been performed on Ps skin. These repeatedly showed a significant up-regulation of IL-36 cytokines, with one study also reporting a correlation between serum IL-36 levels and psoriasis severity [185].

Furthermore, it has been shown that IL-36 has marked pro-inflammatory effects in three-dimensional skin cultures and in mouse models of psoriasis [73], [186], [187]. However, the mechanisms whereby IL-36 up-regulation may contribute to the pathogenesis of Ps have not been fully investigated. The aim of this part of the study was to fill this knowledge gap, by harnessing the power of expression profiling.

The first step was to define an IL-36 signature that could be used to interrogate Ps and GPP transcriptome datasets.

Following treatment of KCs with IL-36α, -β or –γ, marked similarities were observed between the genes that were induced by the three cytokines. Interestingly, this observation was recently confirmed in two independent studies of IL-36 treated KCs [155], [188]. These also shed new light on the mechanism that mediates IL-36 signal transduction, showing a requirement for MyD88 and IκBζ, an atypical member of the IκB family [189].

Of note, the differential expression analysis identified a number of genes that were uniquely induced by IL-36β. Given that the majority of these were non-coding or antisense transcripts, it is tempting to speculate that this cytokine may also play a role in the broader regulation of skin immune homeostasis. In fact, accumulating evidence suggests that lncRNAs are important modulators of the inflammatory response [190]–[193]. Carpenter et al, for example showed that the up-regulation of a specific lncRNA is critical to the modulation of innate immune signalling in mouse [194]. In the context

of skin inflammation, Tsoi et al. identified more than 3,000 human, skin-specific lncRNAs, many of which are co-expressed with immune genes [195]. Thus, it would be interesting to investigate whether the expression of IL-36β-dependent lncRNAs correlates with that of innate immune genes that contribute to KC inflammatory responses.

Following the identification of differentially expressed genes, pathway enrichment analyses were undertaken to investigate the biological processes that are driven by IL-36 cytokines. This identified a very significant over-representation of genes related to *Role of IL-17A in Psoriasis*.

IL-17 is a key disease driver in Ps, as demonstrated by the therapeutic efficacy of the biologics that inhibit its action [196]–[198]. The cytokine is produced by Th17 cells, a T lymphocyte sub-population that differentiates from naïve T-cells exposed to IL-23 [199]. The latter can be secreted by DCs and Langherans cells responding to IL-36 [61], [68]. Thus, IL-36 indirectly promotes IL-17 production by driving the activation of DCs. IL-17, in turn, acts on KCs by inducing the expression of further IL-36 [200]. Therefore, the data presented here supports the existence of a positive feedback loop, driven by the reciprocal up-regulation of IL-17 and IL-36 (Fig. 4.6.1).

IL-36 also contributes to this inflammatory circuit by up-regulating various important chemokines which recruit neutrophils (IL8, S100A7) and Th17 cells (CCL20) to sites of inflammation (Fig. 4.6.1).

Thus, the analyses described in this chapter demonstrated that IL-36 cytokines drive inflammatory pathways which sustain and propagate inflammation in Ps skin. Of note, follow-up work carried out by other members of the Capon lab showed that IL-36 blockade has profound anti-inflammatory effects in an animal model of psoriasis (imiquimod-induced psoriasiform dermatitis), where it reduces epidermal thickness and leukocyte infiltration [201], [202]. Similar results were obtained by other investigators, highlighting IL-36 as a key disease driver and therapeutic target in Ps.

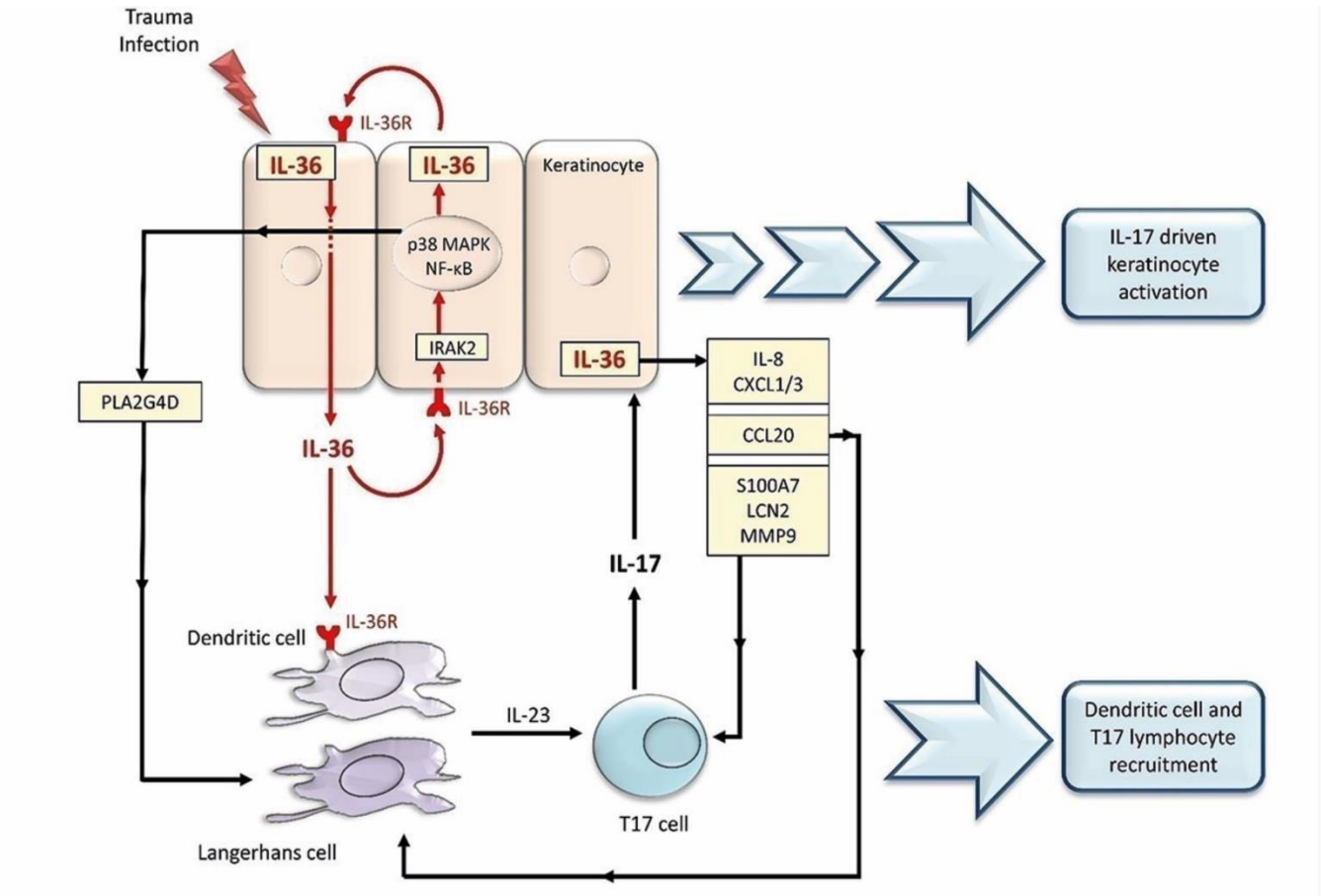


Fig. 4.6. 1.IL-36 enhances skin inflammation by sustaining IL-17 production.

IL-36 cytokines, produced by keratinocytes following trauma or infection, act on dendritic cells and Langerhans cells (via PLA2G4D), resulting in the polarization of Th17 lymphocytes. These release IL-17, which in turn promotes further IL-36 production by keratinocytes.

IL-36 target genes identified by keratinocytes RNA-seq are shown in yellow boxes.

5 The Role of IL-36 in systemic inflammation, a transcriptomic approach

5.1 The whole blood transcription profiles of GPP patients are very heterogeneous

The aim of the second part of the study was to investigate the role of IL-36 cytokines in the systemic pathogenesis of psoriasis. GPP was selected as the initial focus of the investigation given that it manifests with overt systemic symptoms and has been genetically linked to abnormal IL-36 activity.

Whole blood obtained from nine GPP patients and seven healthy individuals was subjected to RNA-sequencing, with a view to determine which transcriptional pathways are disrupted in a condition caused by abnormal IL-36 signalling.

After assessing the quality of the sequence data (Table 5.1.1), raw counts were obtained for ~21,000 genes, including protein coding and non-coding antisense transcripts. In order to visualise this information, dimensionality reduction algorithms were applied. Principal Component Analysis (PCA) showed that the GPP transcriptome is very heterogeneous compared to that of the control group, which forms a better defined cluster (Fig. 5.1.1).

An analysis of possible confounders showed that patients did not cluster by ethnicity, age, mutational status or treatment. In contrast, gender clearly discriminated data distribution along the first two principal components (Fig. 5.1.1) and was therefore included as a covariate when computing differential expression.

Table 5.1.1. Whole-blood RNAseq statistics.

Sample	*Number of paired reads*	*Mapping rate*	*Median raw counts per gene*	*Median RPKM per gene*
C1	15,100,000	95%	54	82.47
C2	19,200,000	98%	73	74.87
C3	19,900,000	98%	92	77.99
C5	19,600,000	98%	88	81.36
C7	18,200,000	98%	87	82.69
C8	20,800,000	97%	98	74.05
C9	18,600,000	95%	84	82.83
GPP1	18,200,000	95%	68	86.31
GPP2	14,800,000	92%	33	87.32
GPP3	19,300,000	88%	59	71.65
GPP4	19,600,000	95%	77	82.00
GPP5	20,200,000	95%	75	87.09
GPP6	21,500,000	95%	58	88.55
GPP7	23,200,000	98%	97	88.18
GPP8	21,200,000	95%	93	82.45
GPP9	18,600,000	90%	92	85.64

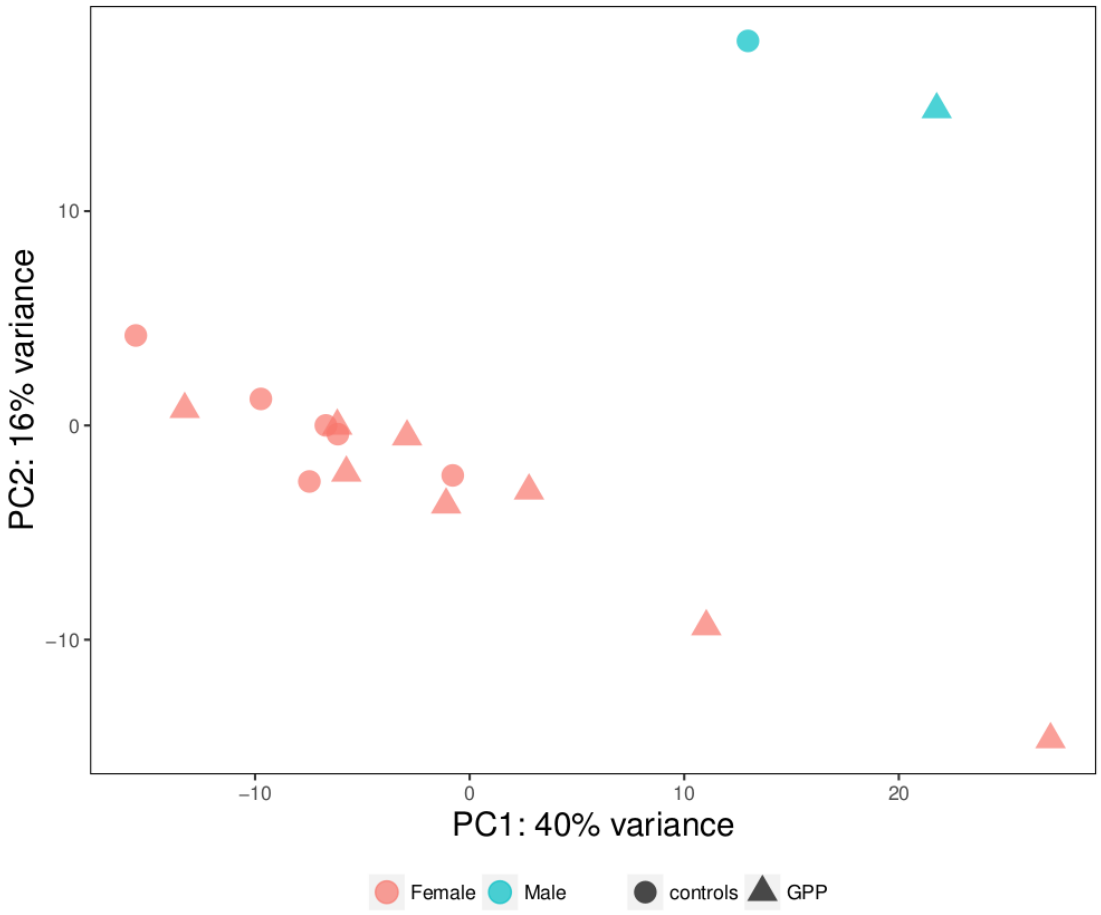


Fig. 5.1.1. Principal component analysis (PCA) of whole-blood RNA-seq data

PCA showing the position of GPP cases (triangles) and healthy individuals (circles). While control samples tend to cluster together, they also overlap with some of the cases. In contrast, males (blue) separate clearly from females (red).

5.2 IL-36 signalling is systemically up-regulated in GPP patients, regardless of mutation status

Only three of the nine patients who were RNA-sequenced harboured *IL36RN* mutations, reflecting the prevalence of *IL36RN* disease alleles in patient cohorts (25-30%) [143]. In this context, it was important to establish that abnormal IL-36 signalling was present in all nine cases.

Five genes that are strongly up-regulated by IL-36 and robustly expressed in whole-blood (*IL1B, PI3, VNN2, TNFAIP6* and *PLSCR1)* were analysed, in order to derive a transcriptional signature of IL-36 activation. Of note, none of the selected genes are up-regulated in the whole-blood of patients suffering from IL-1 mediated autoinflammatory syndromes, indicating that the signature is not influenced by the activation status of IL-1, a cytokine that has powerful systemic effects.

Here, the aggregate expression of the IL-36 signature genes (IL-36 score) was significantly increased in cases compared to controls (P=0.019) (Fig. 5.2.1). Of note, the analysis of a publicly available Ps dataset (whole-blood samples obtained from 33 cases vs 44 controls) [166] identified a moderate, but statistically significant, up-regulation of the same genes (P=0.001) (Fig. 5.2.1). This observation provides a preliminary indication that IL-36 cytokines may also have systemic effects in Ps.

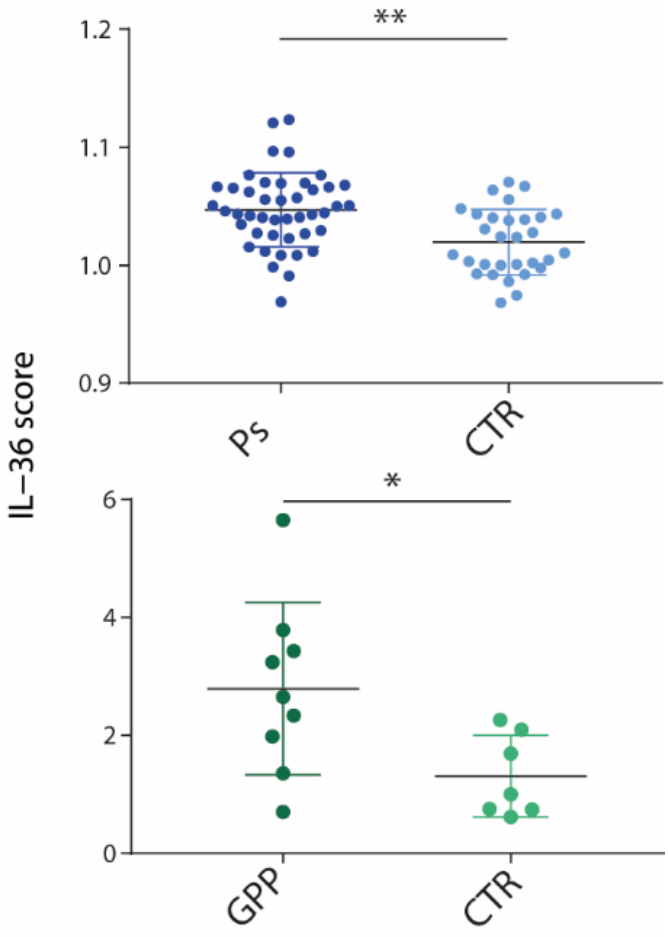


Fig. 5.2.1. The IL-36 score is significantly increased in psoriatic patients compared to controls.

Dot plots showing a higher IL-36 score in whole blood of GPP (green, n=9) or Ps (blue, n=33) patients compared to controls (n=7 or n=44, respectively). The data for each group is presented as mean +/- standard deviation; *P<0.05, **P<0.01 (unpaired t-test).

5.3 Type-I-IFN related pathways are abnormally active in GPP

5.3.1 Genes related to IFN signalling are enriched in the GPP transcriptome

Differential expression analysis of the GPP RNA-seq data identified 117 Differentially Expressed Genes (DEGs), of which 111 were up-regulated (FC≥1.5 and FDR<0.05) (Fig. 5.3.1) (Appendix B).

To better understand the biological significance of these findings, the list of up-regulated genes was used as input for Ingenuity Pathway Analysis (IPA). This revealed 12 enriched pathways (FDR <0.05) (Fig. 5.3.2), the most over-represented of which was *IFN signalling* (FDR = 4.7×10^{-6}).

Other enriched biological processes were also related to innate immunity. These included *IL-6 signalling* (FDR = 1.4×10^{-3}) and *Inflammasome pathway* (FDR = 0.04).

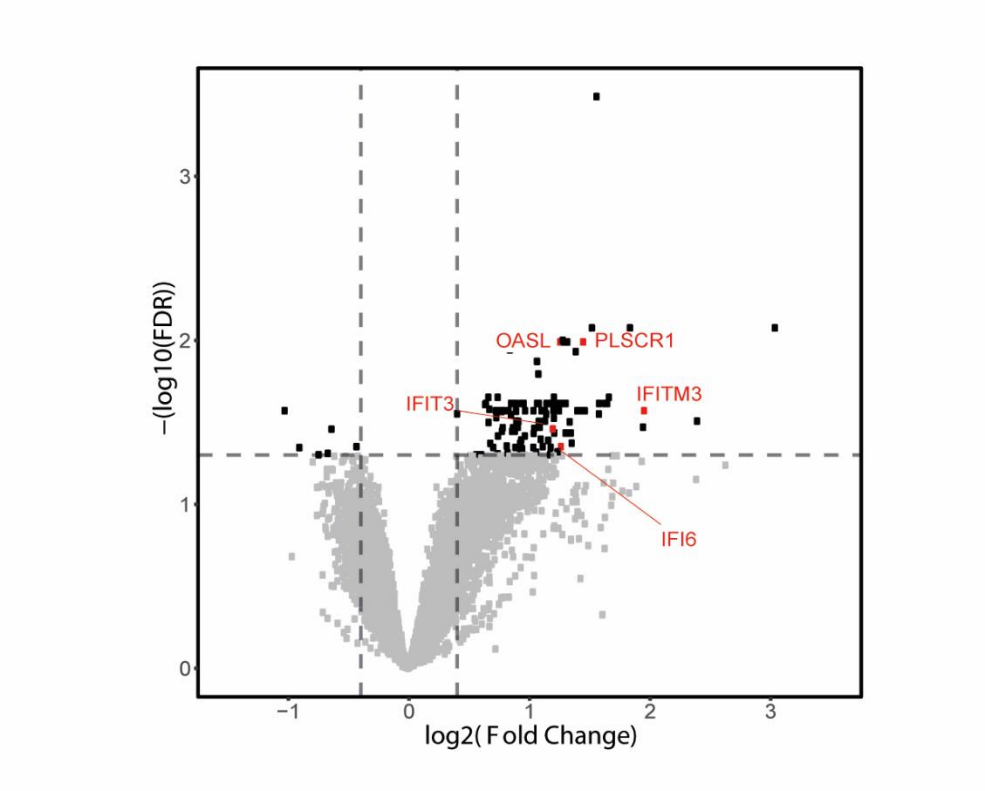


Fig. 5.3.1. Genes that are differentially expressed in the whole blood of GPP patients.

Volcano plot showing differentially expressed genes. The genes meeting the significance threshold (horizontal dashed line, FDR <0.05) and the fold-change threshold (vertical dashed lines, FC>1.5; FC<0.7) are represented as black dots. The genes used to measure the IFN score (see 5.4) are labelled in red font.

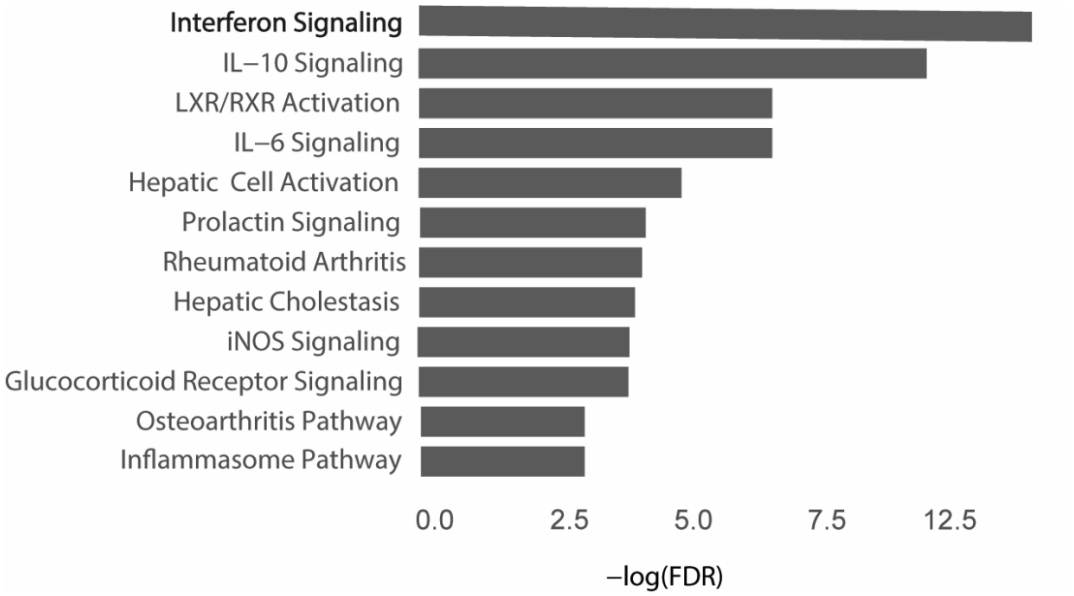


Fig. 5.3.2. Interferon signalling is the most significantly enriched pathway in GPP whole-blood.

Bar plot illustrating the pathways that are enriched (FDR<0.05) among genes up-regulated in GPP (Ingenuity Pathway Analysis).

5.3.2 Type-I IFN regulatory networks are active in the GPP whole-blood transcriptome

To further dissect the role of IFN signalling in the context of GPP, an analysis of blood transcriptional modules (BTMs) was undertaken. BTMs are curated gene networks derived through the analysis of whole blood transcriptomic studies and through text-mining of the links between genes expressed in human blood [177]. Among these modules, only the ones that were active in the GPP RNAseq dataset were considered for further investigation. Their analysis identified nine BTMs that were enriched at an FDR <0.05, among the up-regulated genes in GPP (Fig. 5.3.3).

Of note, all the over-represented modules are related to innate immunity, e.g.: *dendritic cells* and *activated dendritic cells* (FDR = $3.6x10^{-5}$ and $4.7x10^{-5}$, respectively). Moreover, a significant enrichment was observed for *type I Interferon* (FDR = $1.12x10^{-3}$), *innate antiviral response* (FDR = $1.13x10^{-3}$) and *IRF2 target genes* (FDR = $1.19x10^{-3}$), thus confirming the over-representation of IFN-related genes observed with IPA.

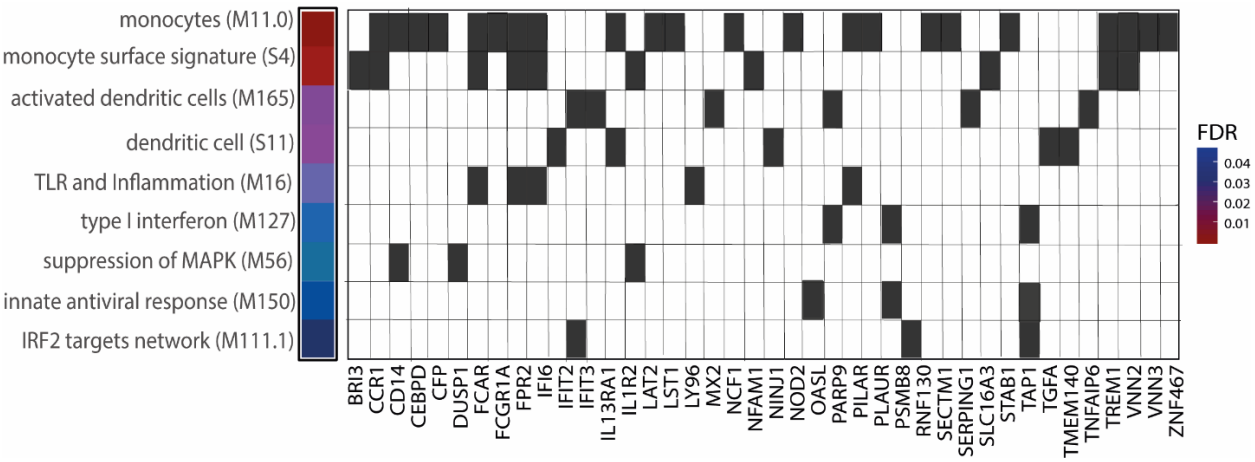


Fig. 5.3.3. Transcriptional modules related to innate responses are enriched among genes over-expressed in GPP.

Diagram showing transcriptional modules that are enriched (FDR <0.05) among genes up-regulated in GPP whole-blood. The heatmap on the left reports the FDR associated with each module. The up-regulated genes mapping to the modules are indicated on the x axis and shown on the grid as grey cells.

5.3.3 INF-α related transcription factors drive gene up-regulation in GPP

As the previous analyses had demonstrated an enrichment of Type-I IFN related pathways, the list of up-regulated genes was further investigated in order to distinguish between the influence of IFN-α and -β signalling.

As the IFN-α and –β pathways differ mainly in terms of upstream drivers [203], [204], the analysis focused on the transcription factors that bind the promoters of up-regulated genes. This identified 114 enriched transcriptional regulators, with STAT3, STAT1 and IRF7 showing the most significant over-representation (FDR $<10^{-13}$) (Fig. 5.3.4). Importantly, IRF7 is constitutively expressed in pDCs where it induces IFN-α expression [205]. Thus, the Type-I IFN signature observed in GPP patients is caused at least in part, by IFN-α up-regulation.

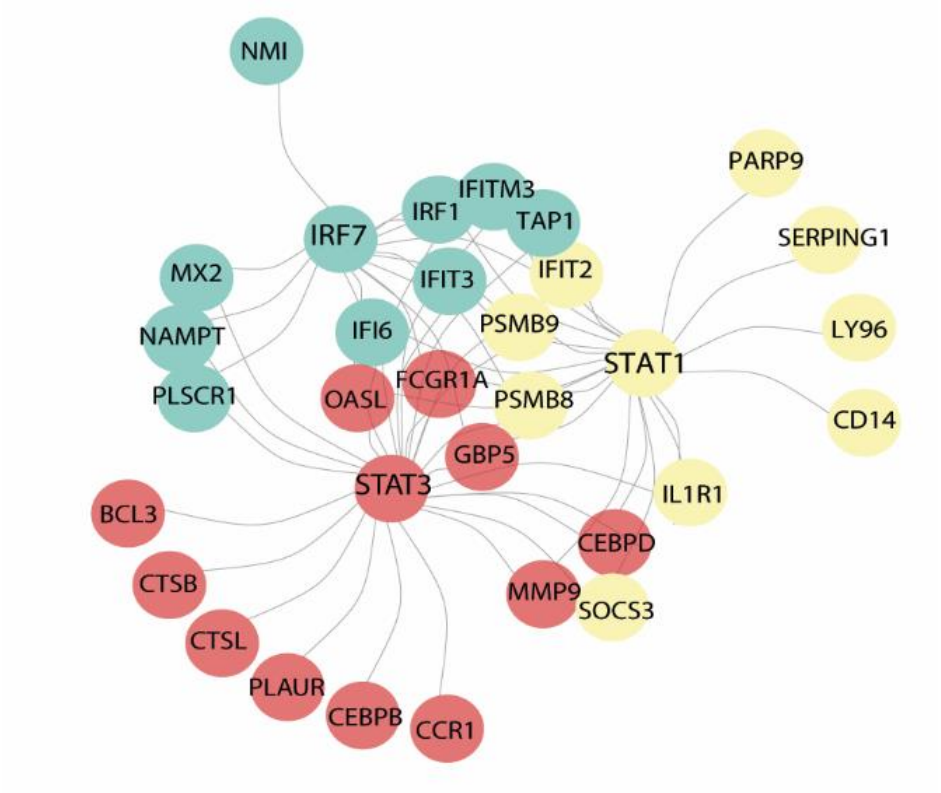


Fig. 5.3.4. Key Type-I IFN regulators driving gene up-regulation in GPP.

Transcriptional networks showing the targets of STAT1, STAT3 and IRF7. All the genes included in the network were up-regulated in GPP (Upsteam Analysis, IPA).

5.4 Overlap between the genes that are up-regulated in GPP and interferonopathies

Abnormal type-I IFN levels are associated with a subset of monogenic autoinflammatory syndromes known as interferonopathies [206]. Interestingly, neutrophilia and systemic inflammation, which are typical manifestations of some of these syndromes (e.g. chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE), Aicardi-Goutiéres syndrome (AGS)), are also key features of GPP.

Here, an analysis of the genes that are over-expressed in CANDLE (8 cases vs 5 controls [167]), showed a significant overlap with those that are up-regulated in GPP ($P < 10^{-10}$) (Fig. 5.4.1). Interestingly, the same did not apply to the genes that are over-expressed in Cryopyrin Associated Periodic Syndrome (CAPS), an IL-1 mediated condition that was analysed as a negative control (P = 0.3) (Fig. 5.4.1).

This is further evidence for a pathogenic role of Type-I IFN signalling in GPP.

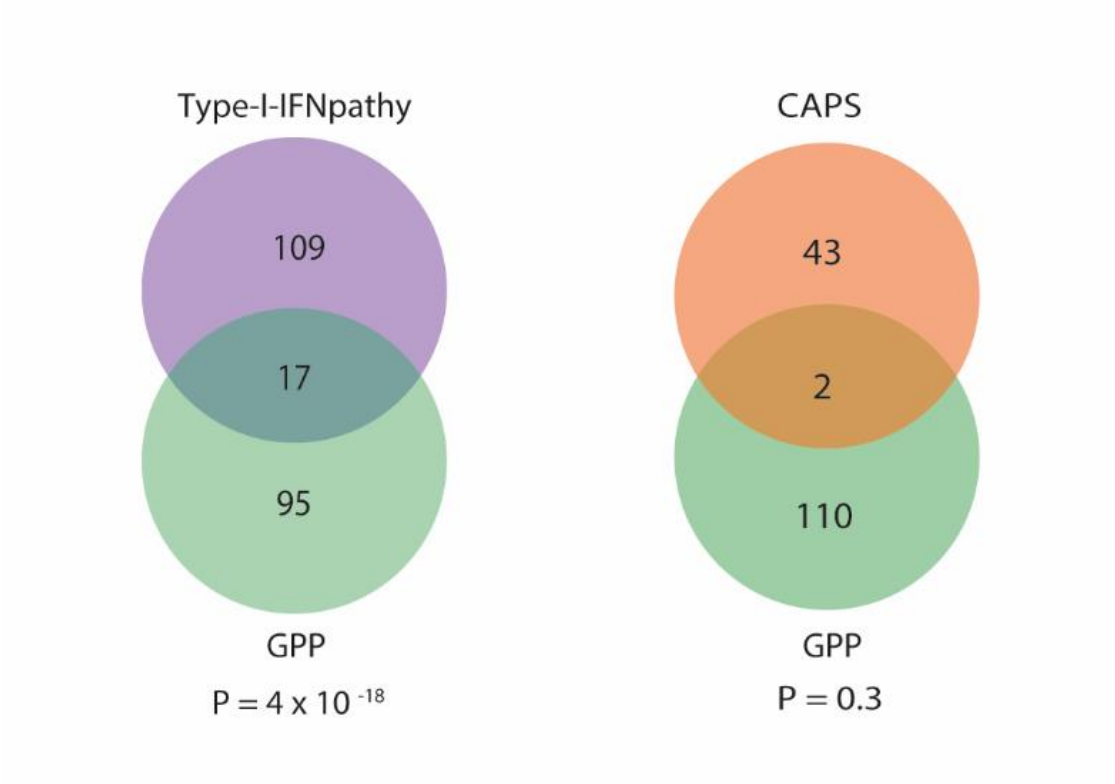


Fig. 5.4.1. Overlap between genes that are over-expressed in GPP and other autoinflammatory diseases.

Venn diagrams showing: the overlap between the genes that are up-regulated in GPP (green) and in the Type-I-IFNpathy known as CANDLE (purple) (left), and the overlap between the genes that are up-regulated in GPP and in the IL-1 driven autoinflammatory disease known as CAPS (orange) (right). P values were computed using a hypergeometric test and statistical significance was validated by bootstrap analysis.

5.5 The IFN score is elevated in psoriatic cases compared to controls

Given the strong type-I IFN signature observed in the GPP transcriptome, one could expect to observe an up-regulation of the genes encoding IFN-α itself. However the presence of numerous gene isoforms and their sequence homology hinder direct measurements of *IFNA* expression [207], [208]. Therefore Type-I IFN levels are typically measured through the analysis of target genes.

Here, an IFN score (IS) was derived by measuring the aggregate expression of five (type-I) interferon inducible genes, as described by Rice et al [178]. In particular, the *IFI6, IFIT3, IFITM3, OASL* and *PLSCR1* genes were selected for analysis, based on their significant up-regulation in GPP whole-blood (Fig. 5.3.1).

As expected, the IS was significantly increased in GPP patients compared to controls (Fig. 5.5.1).

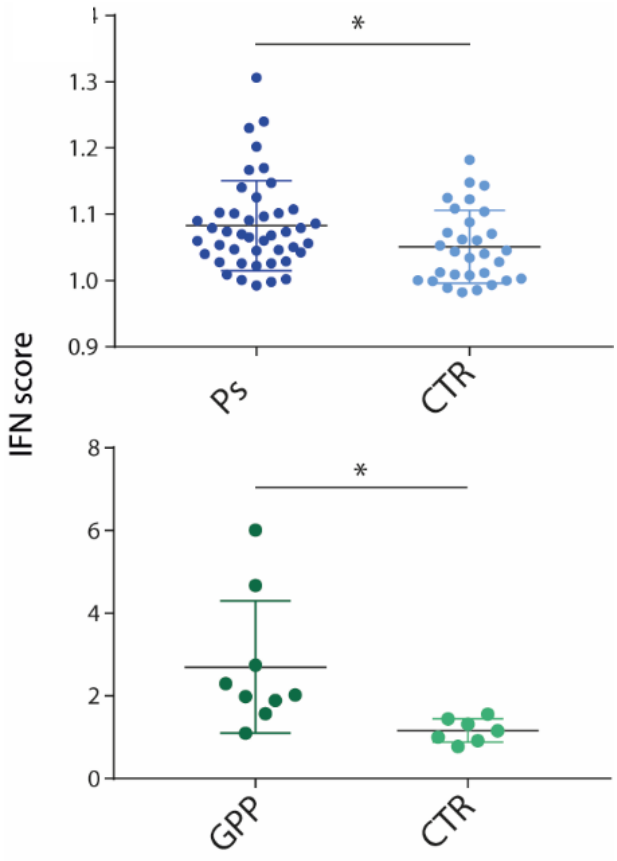


Fig. 5.5.1. The IFN score is elevated in psoriatic patients compared to controls.

Interferon Stimulated Genes show higher expression in the whole blood of GPP (green) and Ps (blue) patients compared to that of healthy controls. Each individual is presented as a dot. Data are shown as mean +/- standard deviation; *P<0.05, **P<0.01 (unpaired t-test).

For validation purposes, whole-blood samples were obtained from 35 additional GPP cases and 7 healthy controls. The expression of the five signature genes was then measured by real-time PCR. The analysis of the expanded dataset (including the validation cohort as well as the samples that had been originally RNA-sequenced) showed a significantly higher score in cases compared to controls (P = 0.02) (Fig.5.5.2).

Given, however, that the examined individuals came from a variety geographical regions, one could question whether this observation was affected by the ethnicity of the study participants. To address this issue, a clustering analysis was undertaken using individuals who had been RNA-sequenced by the 10KImmunome project [165].

This showed that individuals of similar descent do not cluster together when the expression of the five signature genes is examined (Fig. 5.5.3). Moreover, data independently generated by Spielman et al. [209] shows that the expression of the five genes is comparable in European and Asian populations. Thus the IS is unlikely to be influenced by ethnicity and the difference between GPP cases and controls can be considered as genuine.

Importantly, a moderate but statistically significant IS up-regulation was also observed in Ps whole blood (Fig. 5.5.1).

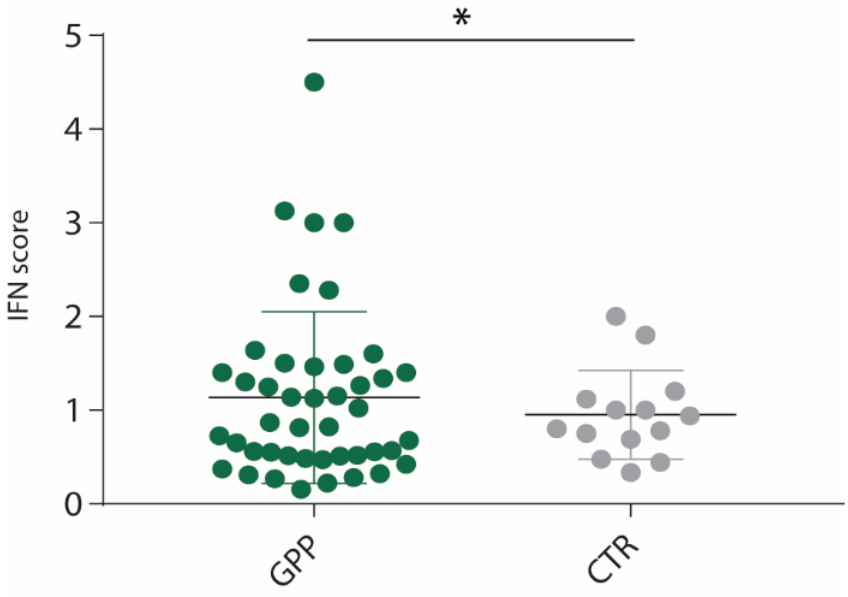


Fig. 5.5.2. **The analysis of an extended dataset validates the abnormal IS observed by RNA-seq.**

Dot plot showing an elevated IFN score in whole-blood samples of GPP patients (expanded dataset, n=44) compared to healthy controls (n=14). The data are presented as mean +/- standard deviation; *P<0.05 (unpaired t-test).

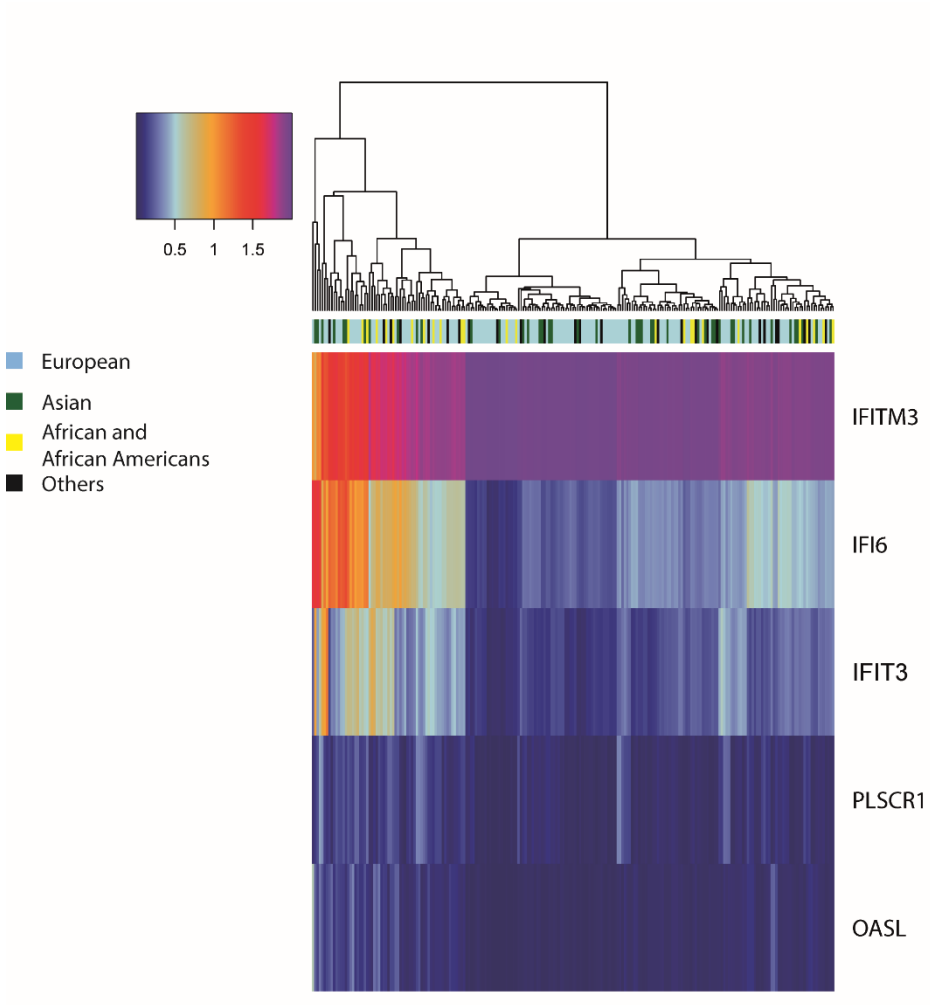


Fig. 5.5.3. Expression of interferon signature genes across individuals of different descent.

Heatmap of gene expression data of the five interferon signature genes (*IFI6*, *IFIT3*, *IFITM3*, *OASL* and *PLSCR1*) in 221 healthy individuals from the 10kImmunome project (44 Asians, 126 Europeans, 25 Africans and African Americans, 26 Others). The dendogram at the top is showing that there is no clustering driven by ethnicity.

5.6 IL-36 and IFN scores are highly correlated

In order to investigate if there is a link between sustained IL-36 signalling and abnormal type-I IFN production, the correlation between IL-36 and IFN scores was measured in both GPP and Ps transcriptomes. The analysis showed a very strong correlation in GPP (rho = 0.93; P = $7.9x10^{-5}$) and a positive correlation in Ps (rho = 0.4; P = $5x10^{-3}$) (Fig 5.6.1). Although this result supports the hypothesis of a link between IL-36 and IFN-α, this needs further investigation and experimental validation.

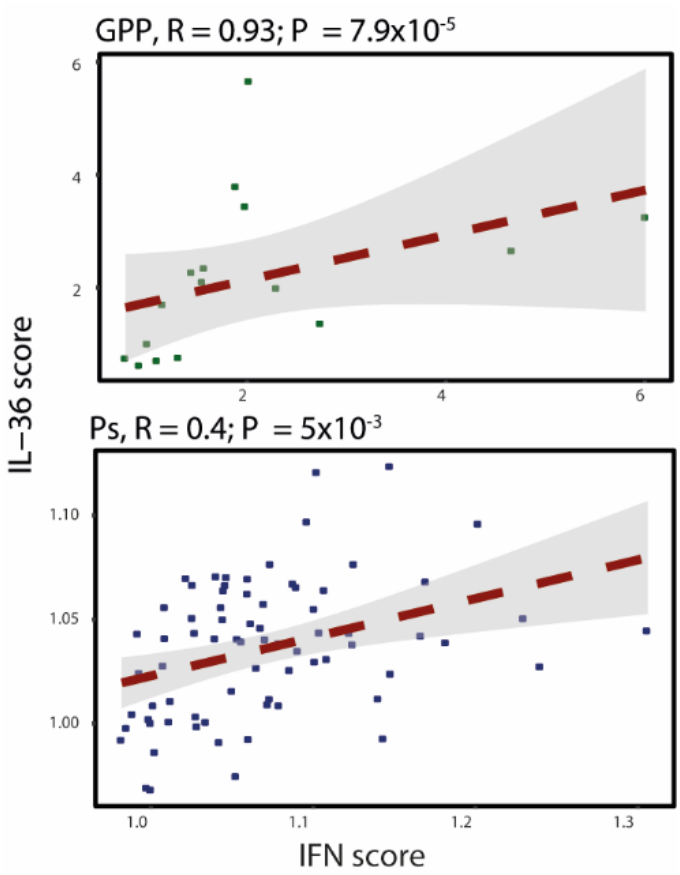


Fig. 5.6.1. IL-36 and IFN scores show significant positive correlation.

Scatter plots showing that IL-36 and IFN scores are significantly correlated, in both GPP (top panel) and Ps (bottom panel) patients. Dashed regression lines are plotted with their 95% confidence intervals (grey areas). Correlation was computed using Spearman's method.

5.7 Discussion

The aim of this part of the project was to explore the role of IL-36 in the inflammatory responses driven by circulating leukocytes.

Several studies described the genetic link between GPP and *IL36RN* mutations [79], [153], [161], [183], [210], [211]. Others have also investigated the role of IL-36 in immune mediated conditions such as psoriasis vulgaris and ulcerative colitis. Thus, the analysis of mouse models and human tissue has shown that IL-36 contributes to epithelial inflammation by promoting the polarization of Th17 cells and up-regulating the production of neutrophil and T cell chemoattractant.

Conversely, the effects of IL-36 on circulating immune cells remain poorly understood. The work described in this chapter sought to address this issue through an investigation of GPP as a model of systemic inflammation caused by abnormal IL-36 signalling.

Firstly, the analysis of whole-blood RNAseq identified an unexpected type-I IFN signature that could be experimentally validated in an extended patient dataset. Interestingly this is consistent with observations published by Wang et al. [19], who found that some loci related to type-I IFN production, such as *IRF7, IFNAR1* and *STAT1*, are down-regulated in PBMCs of GPP patients treated with acitretin.

Moreover, work carried by other members of the group demonstrated that a very significant type-I IFN signature can also be observed in neutrophils purified from the blood of affected individuals. In fact, RNA-sequencing of 8 GPP cases and 11 controls detected a marked over-representation of numerous transcriptional modules related to type-I IFN signalling, viral sensing and innate antiviral responses. These findings complement the results described here and suggest that the type-I IFN signature detected in whole blood is likely to be driven, at least in part, by gene up-regulation in neutrophils.

However, given the manifestations of GPP (e.g. high fever, neutrophilia and high CRP levels) one could conclude that the observed signature is a consequence rather than a cause of systemic inflammation.

To address this possibility, it was important to demonstrate that: i) the GPP transcriptome is not similar to that of other inflammatory diseases presenting with systemic manifestations, and ii) type-I IFN signalling is driven by IL-36 activity in GPP.

In this context, the analysis of publicly available data, showed that there is no meaningful overlap between the genes that are up-regulated in GPP and CAPS. While significant similarities were observed with CANDLE, the majority of over-expressed genes were not shared between the two conditions. These findings suggests that the type-I IFN signature observed in GPP is disease specific and therefore not secondary to its clinical manifestations. Nevertheless, it would be interesting to compare the GPP transcriptome with that of other conditions, such as Crohn's disease and ulcerative colitis, to see if the disease specificity still applies.

While the question relating to the causal role of IL-36 is only partially addressed in this chapter, computational analyses uncovered some interesting clues. First, there was a strong correlation between IL-36 and IFN scores, not only in the original GPP dataset, but also in patients with Ps.

Second, the BTM analysis suggested an involvement of DCs and monocytes, which are the main producers of IL-36 in peripheral blood [69]. Finally, the identification of IRF7 as a transcription factor likely to drive gene up-regulation suggested that IL-36 might have an effect on IFN-α production by pDCs.

In conclusion, the analyses presented in this chapter support the hypothesis that IL-36 contributes to systemic inflammation by up-regulating IFN-α production. However, interferons are produced upon viral infection or as a consequence of infection-like mechanisms [212] and not in response to other cytokines. Thus, various experimental approaches had to be used to reveal the relationship between IL-36 and abnormal type-I IFN levels. The results of these studies are presented in the next chapter.

6 The Role of IL-36 in systemic inflammation, experimental validation

6.1 The IL-36 receptor is robustly expressed on the surface of plasmacytoid dendritic cells

The analysis of GPP whole-blood presented in the previous chapter suggested that there might be a link between IL-36 signalling and type-I IFN production. To explore this hypothesis, it was first necessary to establish what immune cell types can respond to IL-36 stimulation. The expression of the IL-36 receptor (IL-36R) was therefore investigated by flow-cytometry.

PBMCs and neutrophils obtained from healthy individuals and GPP patients were stained with a cocktail of antibodies, targeting IL-36R, as well as cell-specific surface markers.

Interestingly, no receptor expression was observed on T cells, even after activation with CD3/CD28 beads (Fig. 6.1.1). While Arakawa et al. have described a response of T helper cells to IL-36 treatment, our findings are in keeping with those of Fosters et al, who also showed no expression of IL-36R on T cells [61], [120].

Among adaptive immune cells, the strongest IL-36R expression was therefore observed in B cells (Fig. 6.1.1). This was surprising because there is no evidence of B cells being linked to plaque or pustular psoriasis.

Conversely, the highest proportion of innate immune cells expressing IL-36R, was found among pDCs and mDCs, with no IL-36R expression detected on the surface of isolated neutrophils (Fig. 6.1.2). While no significant difference was observed between GPP cases and healthy controls (Fig. 6.1.3), IL-36R levels were especially high in patient pDCs.

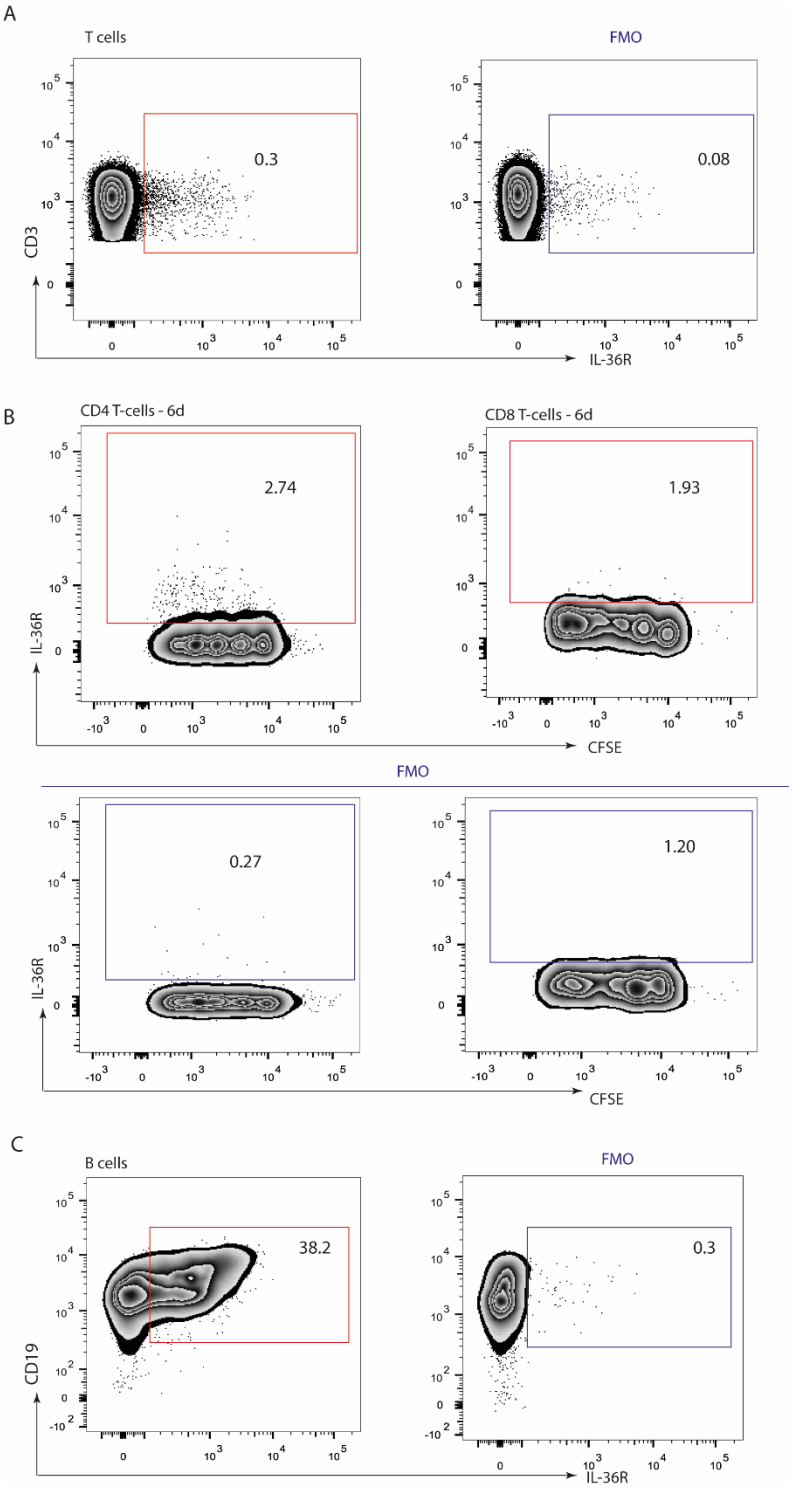


Fig. 6.1.1. IL-36R expression in adaptive immune cells.

(A-C) Representative flow cytometry plots are shown, alongside fluorescence minus one (FMO, blue gates) controls for each population. The following cell types were analysed: (A) T cells (gated as $CD3^+$, $CD19^-$, $CD14^-$ and $CD16^-$); (B) purified T cells cultured for 6 days in the presence of CD3/C28 and gated for CD4 (left) or CD8 (right); (C) B cells ($CD3^-$, $CD14^-$, $CD16^-$, $CD19^+$ and $CD20^+$).

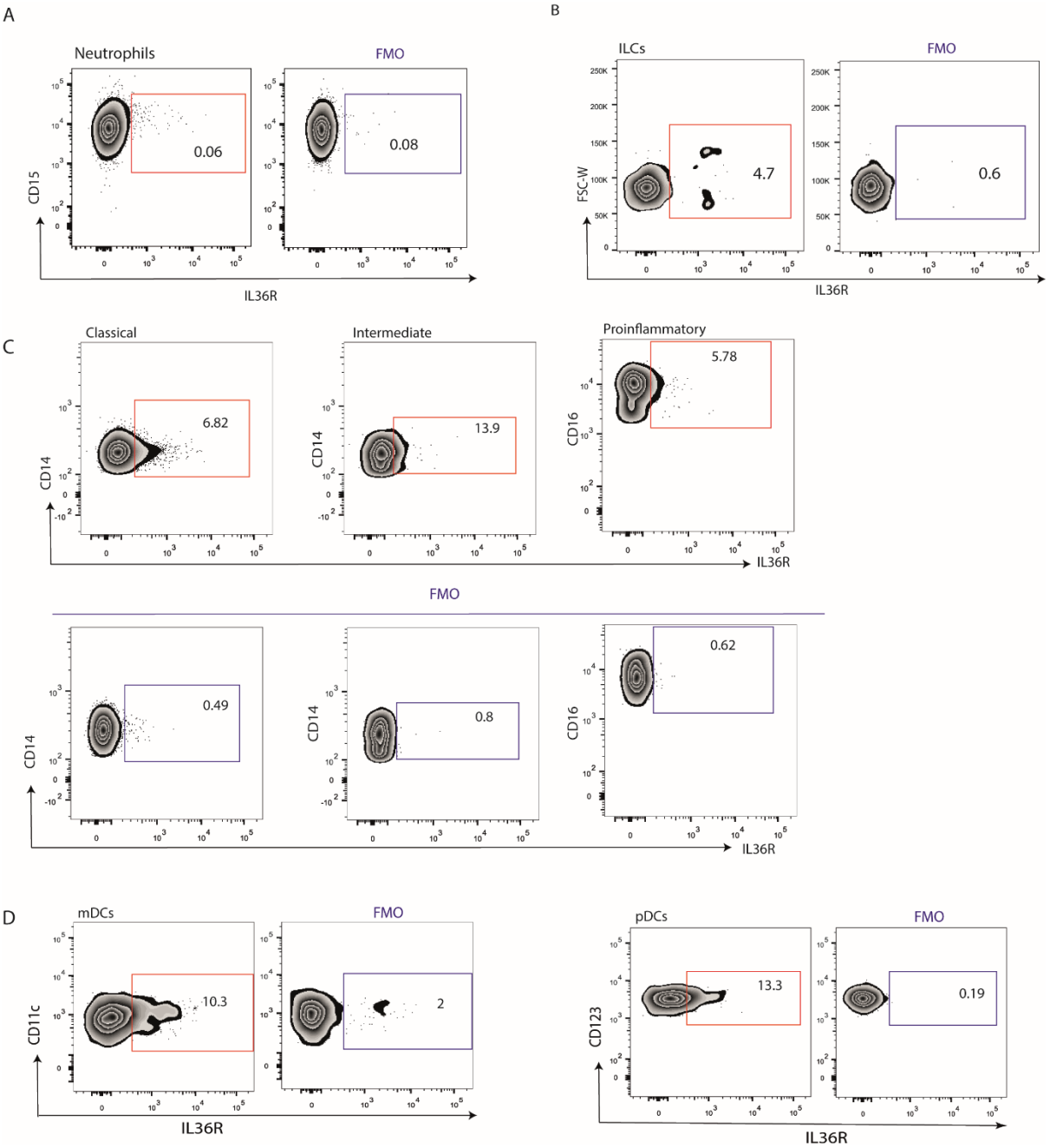


Fig. 6.1. 2. IL-36R expression in innate immune cells.

(**A-D**) Representative flow cytometry plots of IL-36R surface expression in innate immune cells. Fluorescence minus one (FMO, blue gates) controls are shown for each population. The following cell types were analysed: (**A**) purified neutrophils (gated as CD14-, CD15+, CD16+ cells); (**B**) innate lymphoid cells (lineage- (CD3-, CD4-, CD19-, CD20-, CD56-), CD127+); (**C**) monocytes (CD3-, CD20-, CD19-, CD56-) separated into classical (CD16-, $CD14^{high}$), intermediate (CD16+, CD14+) and pro-inflammatory ($CD16^{high}$, CD14-) populations; (**D**) pDCs (lineage-, HLADR+, CD123+, CD11c-) and mDCs (lineage-, HLADR+, CD123-, CD11c+).

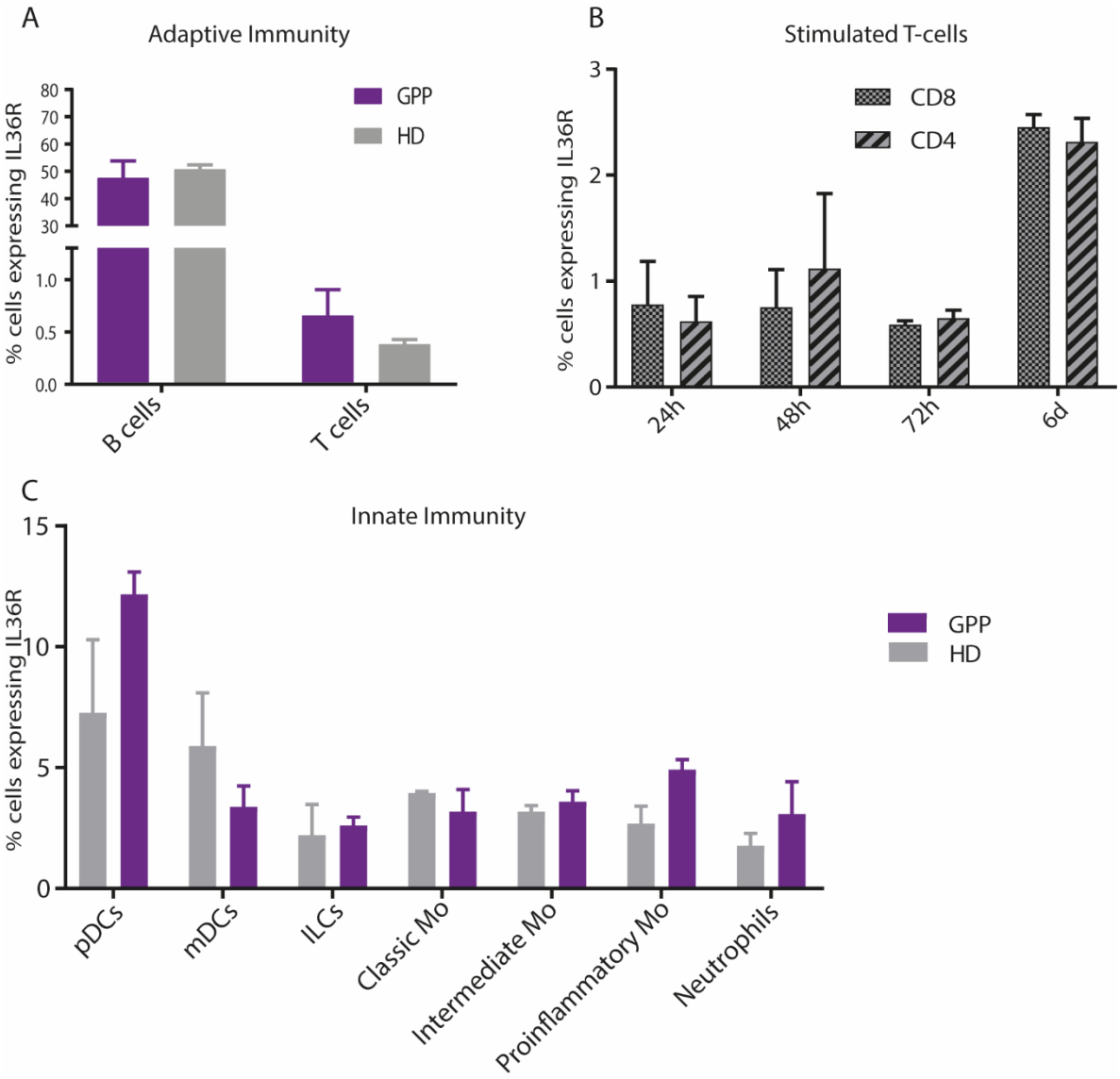


Fig. 6.1.3. IL-36R expression summary data.

Bar plots showing the percentage IL-36R^{+} cells in each leukocyte population (n= 3 GPP cases and n = 3 healthy donors (HD) for PBMCS; n=4 GPP cases and 4 healthy donors for neutrophils). (**A**) Bar plot for B cells and T cells gated as in Fig. 6.1.1. (**B**) Bar plots for purified CD4^{+} and CD8^{+} T cells after 6 days of *in vitro* activation. (**C**) Bar plots for innate immune cells gated as in Fig. 6.1.2. Data are presented as mean +/- SEM.

6.2 IL-36 potentiates IFN-α production

Given that IL-36R is expressed on the surface of pDCs, we hypothesised that IL-36 cytokines potentiate type-I IFN production in these cells. To investigate this possibility, PBMCs obtained from healthy donors were pre-treated with IL-36α, or medium, and then stimulated with CpG-containing DNA (hence CpG), a Toll-like receptor (TLR)-9 ligand which induces IFN-α release by pDCs.

After 6h treatment with CpG, the mRNA expression of the interferon signature genes was markedly increased (Fig. 6.2.1). Interestingly, this up-regulation was more pronounced in cells that had been pre-stimulated with IL-36 ($P<0.05$ for *IFIT3*, *OASL* and *PLSCR1*). These findings were also confirmed at the protein level, as an increase in IFN-α release was observed in the supernatants of cultures pre-treated with IL-36 (Fig. 6.2.1).

To further explore these results, flow cytometry experiments were undertaken in order to identify the cell-type(s) driving IFN-α production. The assays focused on B cells and pDCs, since these showed the highest IL-36R expression.

While IFN-α was not detected in B cells under any conditions, it was present in pDCs stimulated with CpG. Importantly, the proportion of $IFN\alpha^+$ cells was higher among pDCs that had been pre-treated with IL-36 compared to those that had been exposed to CpG alone (Fig. 6.2.2).

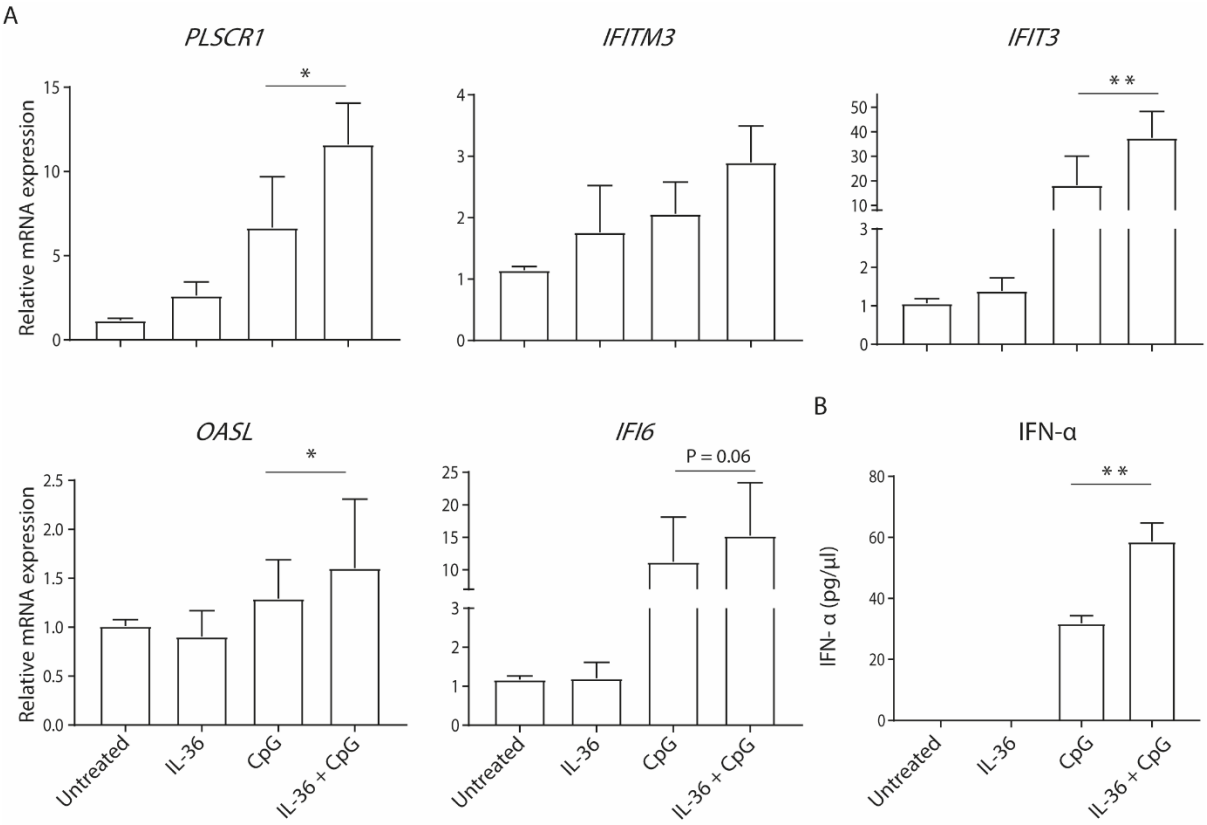


Fig. 6.2. 1. IL-36 pre-treatment enhances IFN-α responses in PBMCs.

(**A**) PBMCs were stimulated with CpG for 6h, in the presence or absence of IL-36 pre-treatment (6h). The expression of the five interferon signature genes was then measured by real-time PCR and normalised to that of the *B2M* gene. Data represent the mean +/- SEM of results obtained in three independent donors, each stimulated in triplicate (**B**) Following PBMC stimulation, IFN-α production was measured by ELISA. Each line in the plot represents an independent healthy donor. Data are plotted as mean+/- SEM.

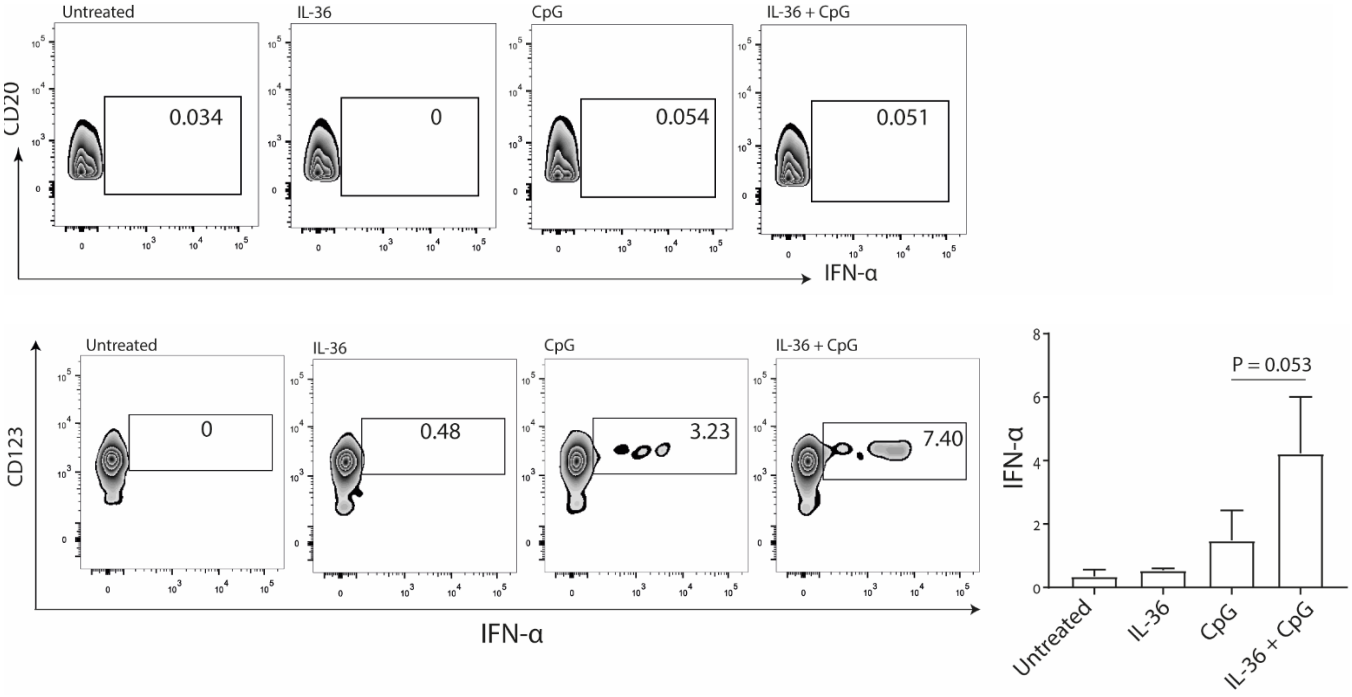


Fig. 6.2. 2. IL-36 potentiates IFN-α production in pDCs but not B cells.

Following PBMCs stimulation, the percentage of IFN-α$^+$ B cells (top panel) and pDCs (bottom panel) was determined by flow cytometry. Representative sets of zebra plots are shown for both populations, while the panel on the bottom right summarizes the data obtained for pDCs (n=3 healthy donors). Data are plotted as mean+/- SEM.*P<0.05; **P<0.01 (Friedman's test, with Dunn's post-test).

6.3 IL-36 up-regulates PLSCR1 expression in pDCs

The above observation suggests that the effect of IL-36 on IFN-α production is mediated by pDCs. Although this is a reasonable conclusion, it does not explain the underlying mechanisms.

Interestingly, an analysis of the results obtained in previous experiments revealed that IL-36 can increase PLSCR1 expression even in the absence of CpG. This result, which was confirmed through the analysis of further donors (Fig. 6.3.1) is noteworthy, since *PLSCR1* encodes a phospholipid scramblase that is responsible for the endosomal translocation of TLR-9. Thus, the up-regulation of PLSCR1 could account for the enhanced TLR-9 activation observed in PBMCs treated with IL-36.

To further explore this possibility, pDCs were isolated from the blood of healthy donors and stimulated with CpG, with or without IL-36 pre-treatment. PLSCR1 expression was then measured by flow cytometry.

As expected, these experiments identified a homogenous pDC population expressing PLSCR1. Importantly, a significant increase in PLSCR1 mean fluorescence intensity (MFI) was observed in IL-36 treated cells compared to untreated (Fig. 6.3.1). This demonstrates that IL-36 acts directly on circulating pDCs, where it up-regulates PLSCR1 mRNA and protein expression.

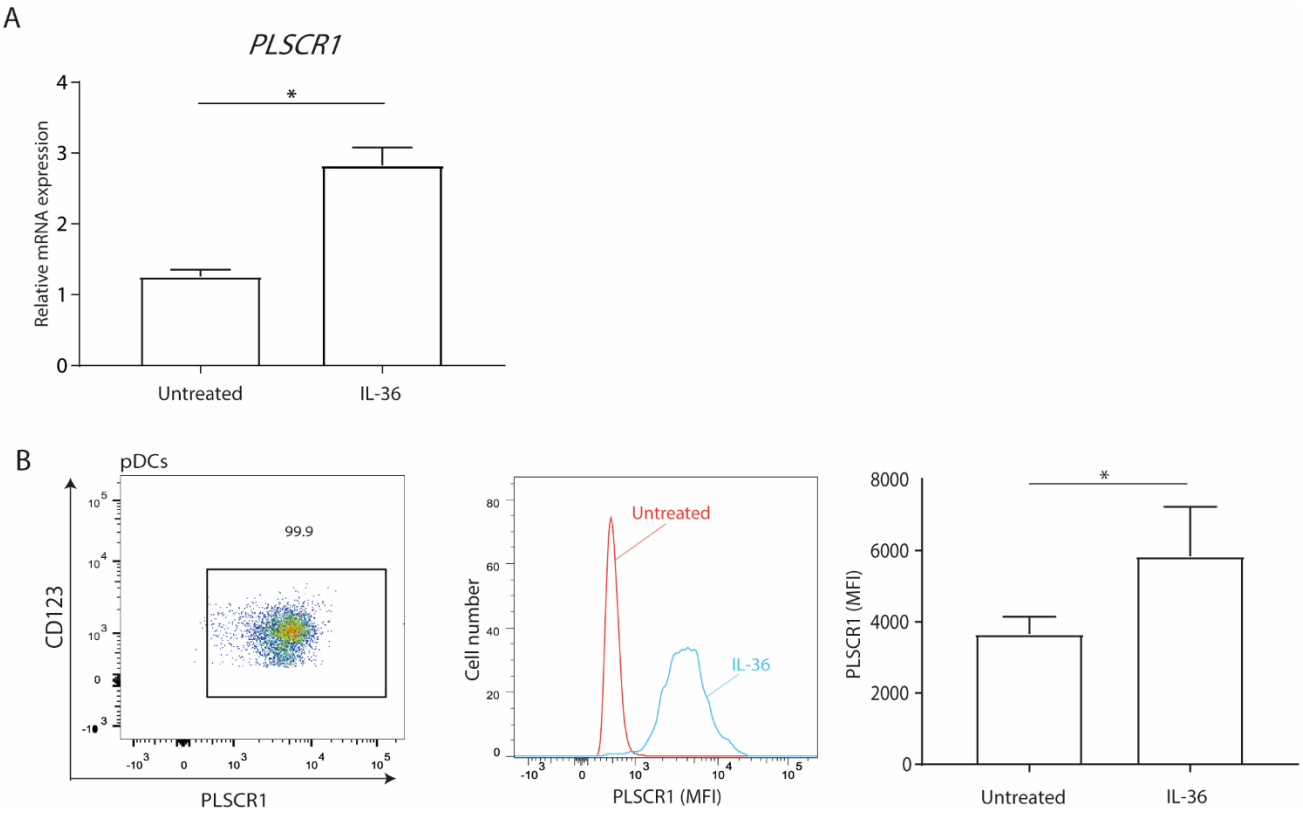


Fig. 6.3.1. IL-36 up-regulates the expression of PLSCR1.

(**A**) Real time qPCR showing up-regulation of *PLSCR1* upon IL-36 stimulation. The bar plot summarises the data obtained in five healthy donors. (**B**) From the left, flow-cytometry gate showing that virtually all pDCs express PLSCR1 following IL-36 treatment. PLSCR1 mean fluorescence intensity (MFI) was measured by flow cytometry. The panel on the left shows a representative histogram (control in orange and treated in blue), while the plot on the right summarises the results obtained in the three healthy donors. Data are plotted as mean+/- SEM. **P*<0.05 (Wilcoxon signed-rank test).

6.4 Discussion

The aim of this chapter was to dissect the molecular mechanisms linking IL-36 signalling to abnormal type-I IFN production. The first step was to identify the cell types that respond to IL-36 stimulation.

No IL-36R expression was observed on T cells, which is in keeping with the results reported by Foster et al [61]. Conversely, Arakawa et al. showed that CD4+ T cells undergo clonal expansion in response to IL-36. This facilitates Th17 polarization and activation, in blood as well as skin.

It is worth noting, however, that the effect observed by Arakawa et al could be an indirect one, as the authors did not show IL-36R expression in circulating T-cells [213]. In fact, the only direct evidence for IL-36R expression in T lymphocytes was shown in the leukocytes infiltrating GPP lesions. This suggests that different tissue-dependent stimuli might induce trafficking of IL-36R on the surface of T cells and that these signals might be mainly active in the skin (rather than the blood) of GPP patients.

Similar considerations may apply to neutrophils. These cells are key mediators of inflammation in GPP, as they infiltrate skin pustules and produce pro-inflammatory cytokines [183], [214]. Nevertheless, circulating neutrophils do not express IL-36R and other members of the group have shown that they do not respond to IL-36 stimulation. Of note, Wang et al have reported that IL-36R is expressed in the neutrophils that infiltrate the polyps of chronic rhinosinusitis patients [53]. Thus, the existence of tissue-specific factors influencing IL-36R expression has also been demonstrated for neutrophils.

The highest percentage of IL-36R$^+$ cells was observed among B lymphocytes. This is in agreement with the data reported by Chu et al., who detected IL-36R expression on regulatory and CD19$^+$B cells [215].

Of note, a role of B cells in innate immunity has been emerging in recent years. Tonti E. et al, for example showed that B cells can be activated by NK cells in a T-cell-independent fashion [216]. An innate-like function has also been described for marginal zone B cells, as these can mount rapid antibody responses that require germline-encoded receptors [217]. Given that preliminary results

obtained by the Capon group suggest that IL-36R is strongly expressed in circulating marginal zone B-cells, it will be interesting to further investigate the role of this subset in autoinflammation.

Here, follow-up experiments were focused on pDCs, as a substantial proportion of these cells, which are major IFN-α producers, expressed IL-36R.

Real-time and flow cytometry experiments demonstrated that IL-36 up-regulates PLSCR1 in pDCs. Given that IL-36 can signal through mitogen-activated protein kinases (MAPK) [218], the latter pathway is likely to contribute PLSCR1 up-regulation, through a cross-talk with STAT1 signalling. In fact, other members of our group have shown that treatment of PBMCs with a MAPK inhibitor abolishes the effects of IL-36 on PLSCR1 mRNA levels.

Interestingly, Talukder et al. reported that primary pDCs from Plscr1-deficient mice produced less IFN-α in response to CpG than pDCs from wild-type mice [219]. This was due to the fact that Plscr1 KO mice were not able to recruit TLR-9 to the endosome of pDCs. As a consequence, there was no downstream signalling leading to the transcriptional activation of IFNA genes.

Our experiments show that IL-36 may have an opposite effect, up-regulating PLSCR1 expression and therefore potentiating TLR-9 activation and IFN-α production.

In conclusion, the findings described in this chapter identify a new IL-36/IFN-α axis that is active in the pDCs of psoriatic patients. More specifically, the data suggests that IL-36 cytokines produced by mDCs have a priming effect on pDCs. Thus, when the latter are exposed to a viral infection or viral-like trigger (e.g. nucleic acids released by apoptotic cells), they produce excessive amounts of IFN-α. Importantly, type-IFN can in turn up-regulate PLSCR1 expression, driving a pro-inflammatory feedback loop in pDCs (Fig. 6.5.1).

Given that IL-36 signalling is abnormally active in the blood of Ps and GPP patients, IL-36 driven IFN-α up-regulation is likely to contribute to the pathogenesis of both conditions. While additional studies will be required to dissect the consequences of abnormal Type-I IFN activity in these diseases, it is

worth pointing out that IFN-α and -β have been repeatedly implicated in the pathogenesis of co-morbidities such as psoriatic arthritis and atherosclerosis [74], [220]. So, the immune axis described here might in the future be targeted for the treatment of extra-cutaneous manifestation of psoriasis.

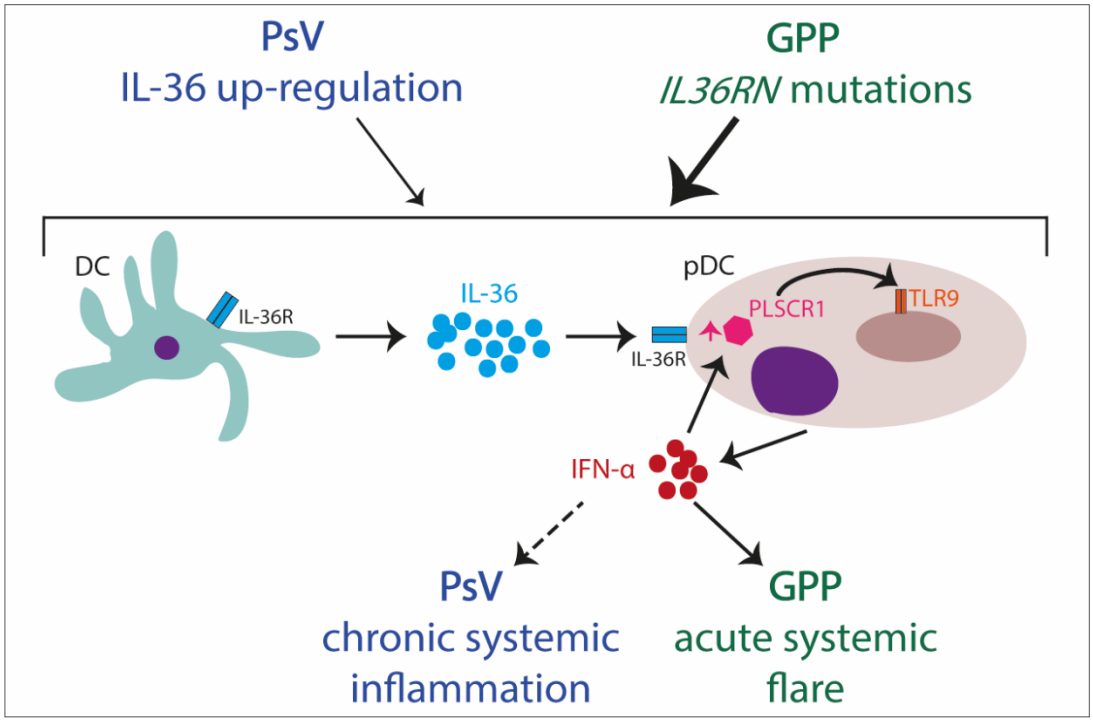


Fig. 6.4.1. IL-36/IFN-α axis: proposed model.

IL-36 produced by mDCs up-regulates *PLSCR1* expression in pDCs, enhancing IFN-α release via TLR-9. IFN-α, in turn, induces further *PLSCR1* transcription, thus promoting a positive feedback loop.

7 Discussion

While numerous studies have documented an up-regulation of IL-36 in psoriatic skin [61], [221, p. 36], the mechanisms whereby this cytokine contributes to disease pathogenesis have mostly been investigated in mouse models [157], [198]. The latter, however, do not recapitulate some of the key features of psoriasis (e.g. the chronic nature of the condition). Thus, the aim of this study was to generate new insights into the pathogenic role of IL-36, by applying transcriptomic approaches to the analysis of human tissues.

7.1 Transcriptomic to unravel mechanisms of inflammatory diseases

RNA-seq is an advantageous tool for the study of gene expression patterns in different organisms and tissues. A key feature in the design of these experiments is the selection of a cell type that is relevant to the disease of interest. In the context of plaque psoriasis, for example, keratinocytes are often used as a model [187], [222]. Since they are the most abundant cell-type in the epidermis and show robust IL-36R expression, keratinocytes have also been examined in this thesis, with the purpose of investigating the consequences of IL-36 up-regulation in psoriatic skin.

While the analysis of a single cell type allowed the definition of a very specific IL-36 signature, it is important to bear in mind that skin is a heterogeneous tissue, characterised by the cross-talk between keratinocytes, fibroblasts and resident immune cells [223]. To better understand the role of IL-36 in this complex environment, future studies should investigate the effects of the cytokine on cultured skin biopsies or infiltrating leukocytes isolated from patient lesions.

In the second part of the study, the systemic effects of IL-36 were examined through the analysis of circulating immune cells. While its ease of access makes blood an attractive choice for transcription profiling experiments, the need to eliminate globin transcripts can affect RNA quality (in our hands, RIN values dropped by as much as 15% following globin depletion). Blood is also the human tissue

with the lowest median gene expression [224]. This affects signal to noise ratios and often reduces statistical power, as demonstrated by the relatively small number of DEGs detected in chapter 4 (< 200).

Despite the limitations inherent to the choice of particular tissues, transcription profiling can offer important insights into disease mechanisms. This is especially the case when the identification of DEG is followed by the study of transcriptional networks or enriched pathways. Of note, the algorithms that are applied in these follow-up analyses rely on the availability of functional annotation for the genes of interest. While several databases have collated information on human genes (e.g. Gene Ontology [104], DAVID [225],Molecular Signature Database (MSigDB) [226] and IPA (Qiagen)), up to 50% of human transcripts cannot be annotated with functional terms. This knowledge gap can sometimes be filled with a guilt-by-association approach, whereby gene function is inferred based on the annotation of co-expressed genes (e.g. GeneMania) [227], [228]. However, the depth of these predicted annotations is variable.

Transcriptomic studies can also offer pathogenic insights by facilitating the identification of non-coding disease alleles. For instance, Cummings et al recently integrated whole-genome sequencing and RNA-seq to identify splice-altering mutations in deep intronic regions [229]. Likewise, Kremer et al demonstrated the advantage of transcriptome sequencing in the molecular diagnosis of mitochondrial disease [230]. Given that the GPP genes described so far (*IL36RN*, *CARD14* and *AP1S3*) only account for a minority of disease cases (~25%), the whole-blood expression profiles generated in this study could be queried with tools that allow the identification of abnormal transcripts isoforms (e.g. *de novo* transcript assembly). These could then be linked to non-coding sequence changes that are predicted to affect splicing enhancers and silencers.

Finally, the development of single-cell RNAseq (scRNA-seq) has further expanded the scope of transcriptomic studies and their potential to illuminate disease pathways [231]. Regulatory networks and their genetic determinants can also be investigated at a resolution that was until recently

impossible, as exemplified by the recent discovery of the first quantitative trait loci for co-expression [232]. In this context, the Capon lab has recently undertaken the scRNAseq of PBMCs stimulated with IL-36. These experiments have the potential to further dissect the pro-inflammatory networks that are activated by this cytokine and up-regulated in psoriatic patients.

7.2 The pathogenic role of IL-36

The work presented in this thesis (chapter 4) demonstrates that IL-36 is a key driver of psoriatic inflammation, as it amplifies the effects of IL-17 signalling in skin. This has now been confirmed in a number of independent studies. For example, German et al have shown that calcipotriol (a vitamin D3 analogue commonly used for the treatment of psoriasis) relieves skin inflammation by down-regulating IL-36 levels and consequently reducing IL-17 activation [233]. Mercurio et al have also reported that an imbalance between IL-36 and IL-38 (an immunoregulatory cytokine that is a paralogue of IL-36Ra) is associated with increased psoriasis severity [234].

Of note, additional associations between IL-36 and pustular skin phenotypes have also been described. For example, it has been shown that the culprit drugs causing Acute Generalised Exanthematous Pustulosis (a severe drug reaction mimicking the presentation of GPP) trigger the production of IL-36 in cultured patient keratinocytes [21].

Thus, the role of IL-36 in skin inflammation has now been investigated in a variety of diseases and experimental systems. Conversely its systemic effects have received little attention.

Here, the analysis of whole-blood gene expression in GPP cases vs. healthy individuals has led to the discovery of a new IL-36/type-I IFN axis. Of note, IL-36 scores were elevated in all patients, even though *IL36RN* mutations were only found in a minority. This supports the notion that genetic defects and disease pathways converge on the up-regulation of IL-36 signalling.

Follow-up mechanistic studies showed that IL-36R expression was highest among pDCs and B cells. The effects of IL-36 on pDCs was further explored and linked to the up-regulation of PLSCR1 and activation of TLR-9 signalling.

While the role of IL-36 in B cell biology was not investigated in this study, another member of the Capon group has since examined the expression of IL-36R in B cell sub-populations. These experiments have shown that IL-36R levels are highest in naïve mature and Marginal Zone B cells. The latter are innate-like B lymphocytes, which rapidly acquire immune regulatory activities through the secretion of natural IgM and IL-10 [236]. They might therefore have a role in breaking self-tolerance and, potentially, in the development of immune related disorders.

Importantly, abnormal IL-36 activity has now been detected in other inflammatory diseases beyond psoriasis. In fact, studies of animal models have repeatedly implicated IL-36 in the pathogenesis ulcerative colitis, whereas measurement of serum cytokine levels have suggested a link with systemic lupus erythematosus [84], [215]. Given the role of pDCs and B cells in the above conditions, future studies should seek to determine whether IL-36 mediated activation of these cell types contributes to disease pathogenesis.

7.3 IL-36 as a therapeutic target in psoriasis

IL-36 signalling inhibition is emerging as a safe alternative to available treatments for Ps and GPP. For instance, Mahil et al showed that individuals born without a functioning IL-36R (human IL1RL2 knockouts) are healthy and do not show an increased susceptibility to infections [20]. These observations suggested that IL-36 blockade is unlikely to have major adverse consequence on human immune function. This notion was subsequently confirmed in clinical trials of IL-36R blockers.

AnaptysBio recently carried out a placebo-controlled, Phase 1 trial of ANB019, a neutralising, anti-IL36R antibody (ClinicalTrials.gov Identifier: NCT03619902). This showed that IL-36R blockade is well tolerated. Only minor side effects were observed, with mild infections of the upper respiratory tract seen in 28% of subjects receiving the drug.

Following these encouraging results, a Phase 2 clinical trial of ANB019 has been initiated in Generalized Pustular Psoriasis (NCT03633396) [25]. Of note, the study is being led from St John's Institute of Dermatology, where skin and blood samples are actively being collected before and after treatment. Plans are in place for the transcription profiling of these samples, which is expected to yield further insights into the pathogenic role of IL-36 and the immunological effects of its blockade.

A parallel phase I trial of a second IL-36R inhibitor (BI 655130) has recently been completed by Boheringer Ingelheim (NCT03482635). The study included a small number of GPP patients (n=7), where complete, or near complete clearance of skin lesions was observed, regardless of IL36RN mutation status [25]. The same agent is now being tested in ulcerative colitis and palmar plantar pustulosis.

While the studies described above included a small number of participants, they seem to consistently show that IL-36 blockers are safe and beneficial. In this context, further studies of the immune pathways and diseases that are affected by IL-36 de-regulation have the potential to identify other disorders that could be treated with this new class of biologics.

8 References

[1] T. J. Kindt, R. A. Goldsby, B. A. Osborne, and J. Kuby, *Kuby immunology*, 6th ed. New York: W.H. Freeman, 2007.

[2] G. Dranoff, "Cytokines in cancer pathogenesis and cancer therapy," *Nature Reviews Cancer*, vol. 4, no. 1, pp. 11–22, Jan. 2004.

[3] B. Alberts, Ed., *Molecular biology of the cell*, 4th ed. New York: Garland Science, 2002.

[4] X. Kong, Z. Yuan, and J. Cheng, "The function of NOD-like receptors in central nervous system diseases," *J. Neurosci. Res.*, vol. 95, no. 8, pp. 1565–1573, 2017.

[5] F. Martinon, A. Mayor, and J. Tschopp, "The inflammasomes: guardians of the body," *Annu. Rev. Immunol.*, vol. 27, pp. 229–265, 2009.

[6] T. Kawai and S. Akira, "The roles of TLRs, RLRs and NLRs in pathogen recognition," *Int. Immunol.*, vol. 21, no. 4, pp. 317–337, Apr. 2009.

[7] R. Medzhitov, "Recognition of microorganisms and activation of the immune response," *Nature*, vol. 449, no. 7164, pp. 819–826, Oct. 2007.

[8] A. Iwasaki and R. Medzhitov, "Control of adaptive immunity by the innate immune system," *Nature Immunology*, vol. 16, no. 4, pp. 343–353, Apr. 2015.

[9] E. Kolaczkowska and P. Kubes, "Neutrophil recruitment and function in health and inflammation," *Nature Reviews Immunology*, vol. 13, no. 3, pp. 159–175, Mar. 2013.

[10] D. Gutowska-Owsiak and G. S. Ogg, "The epidermis as an adjuvant," *J. Invest. Dermatol.*, vol. 132, no. 3 Pt 2, pp. 940–948, Mar. 2012.

[11] M. Yamamoto and K. Takeda, "Current Views of Toll-Like Receptor Signaling Pathways," *Gastroenterology Research and Practice*, vol. 2010, pp. 1–8, 2010.

[12] A. McDermott *et al.*, "A case of proteasome-associated auto-inflammatory syndrome with compound heterozygous mutations," *Journal of the American Academy of Dermatology*, vol. 69, no. 1, pp. e29–e32, Jul. 2013.

[13] D. McGonagle and M. F. McDermott, "A Proposed Classification of the Immunological Diseases," *PLoS Medicine*, vol. 3, no. 8, p. e297, Aug. 2006.

[14] T. Bachetti *et al.*, "Autophagy contributes to inflammation in patients with TNFR-associated periodic syndrome (TRAPS)," *Ann. Rheum. Dis.*, vol. 72, no. 6, pp. 1044–1052, Jun. 2013.

[15] I. Aksentijevich *et al.*, "The clinical continuum of cryopyrinopathies: Novel CIAS1 mutations in North American patients and a new cryopyrin model," *Arthritis & Rheumatism*, vol. 56, no. 4, pp. 1273–1285, Apr. 2007.

[16] I. Aksentijevich *et al.*, "An Autoinflammatory Disease with Deficiency of the Interleukin-1–Receptor Antagonist," *New England Journal of Medicine*, vol. 360, no. 23, pp. 2426–2437, Jun. 2009.

[17] C. A. Dinarello, A. Simon, and J. W. M. van der Meer, "Treating inflammation by blocking interleukin-1 in a broad spectrum of diseases," *Nature Reviews Drug Discovery*, vol. 11, no. 8, pp. 633–652, Aug. 2012.

[18] F. Martinon and I. Aksentijevich, "New players driving inflammation in monogenic autoinflammatory diseases," *Nature Reviews Rheumatology*, vol. 11, no. 1, pp. 11–20, Jan. 2015.

[19] A. Brehm *et al.*, "Additive loss-of-function proteasome subunit mutations in CANDLE/PRAAS patients promote type I IFN production," *Journal of Clinical Investigation*, vol. 125, no. 11, pp. 4196–4211, Oct. 2015.

[20] A. Brehm and E. Krüger, “Dysfunction in protein clearance by the proteasome: impact on autoinflammatory diseases,” *Semin Immunopathol*, vol. 37, no. 4, pp. 323–333, Jul. 2015.
[21] A. A. de Jesus *et al.*, “Novel proteasome assembly chaperone mutations in PSMG2/PAC2 cause the autoinflammatory interferonopathy CANDLE/PRAAS4,” *J. Allergy Clin. Immunol.*, Jan. 2019.
[22] Y. J. Crow, “Type I interferonopathies: Mendelian type I interferon up-regulation,” *Current Opinion in Immunology*, vol. 32, pp. 7–12, Feb. 2015.
[23] M. Bienias *et al.*, “Therapeutic Approaches to Type I Interferonopathies,” *Curr Rheumatol Rep*, vol. 20, no. 6, p. 32, 20 2018.
[24] M. D. Cooper and M. N. Alder, “The Evolution of Adaptive Immune Systems,” *Cell*, vol. 124, no. 4, pp. 815–822, Feb. 2006.
[25] M. L. Dustin, “The Cellular Context of T Cell Signaling,” *Immunity*, vol. 30, no. 4, pp. 482–492, Apr. 2009.
[26] H. Chiba *et al.*, “Cloning of a Gene for a Novel Epithelium-specific Cytosolic Phospholipase A $_2$, cPLA $_2$ δ, Induced in Psoriatic Skin,” *Journal of Biological Chemistry*, vol. 279, no. 13, pp. 12890–12897, Mar. 2004.
[27] K. L. Cheung *et al.*, “Psoriatic T cells recognize neolipid antigens generated by mast cell phospholipase delivered by exosomes and presented by CD1a,” *The Journal of Experimental Medicine*, vol. 213, no. 11, pp. 2399–2412, Oct. 2016.
[28] T. R. Mosmann, H. Cherwinski, M. W. Bond, M. A. Giedlin, and R. L. Coffman, “Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins,” *J. Immunol.*, vol. 136, no. 7, pp. 2348–2357, Apr. 1986.
[29] O. Kaminuma *et al.*, “T-box 21 transcription factor is responsible for distorted TH2 differentiation in human peripheral CD4+ T cells,” *Journal of Allergy and Clinical Immunology*, vol. 123, no. 4, pp. 813-823.e3, Apr. 2009.
[30] L. Steinman, “Mixed results with modulation of TH-17 cells in human autoimmune diseases,” *Nature Immunology*, vol. 11, no. 1, pp. 41–44, Jan. 2010.
[31] E. Schmitt, M. Klein, and T. Bopp, “Th9 cells, new players in adaptive immunity,” *Trends in Immunology*, vol. 35, no. 2, pp. 61–68, Feb. 2014.
[32] V. Dardalhon *et al.*, “IL-4 inhibits TGF-β-induced Foxp3+ T cells and, together with TGF-β, generates IL-9+ IL-10+ Foxp3− effector T cells,” *Nature Immunology*, vol. 9, no. 12, pp. 1347–1355, Dec. 2008.
[33] A. Mohr, M. Atif, R. Balderas, G. Gorochov, and M. Miyara, “The role of FOXP3+ regulatory T cells in human autoimmune and inflammatory diseases,” *Clin. Exp. Immunol.*, Mar. 2019.
[34] R. N. Germain, “T-cell development and the CD4–CD8 lineage decision,” *Nature Reviews Immunology*, vol. 2, no. 5, pp. 309–322, May 2002.
[35] E. H. Meyer, R. H. DeKruyff, and D. T. Umetsu, “T Cells and NKT Cells in the Pathogenesis of Asthma,” *Annual Review of Medicine*, vol. 59, no. 1, pp. 281–292, Feb. 2008.
[36] D. L. Northrup and D. Allman, “Transcriptional regulation of early B cell development,” *Immunologic Research*, vol. 42, no. 1–3, pp. 106–117, Oct. 2008.
[37] T. W. LeBien and T. F. Tedder, “B lymphocytes: how they develop and function,” *Blood*, vol. 112, no. 5, pp. 1570–1580, Sep. 2008.
[38] J. C. Cambier, S. B. Gauld, K. T. Merrell, and B. J. Vilen, “B-cell anergy: from transgenic models to naturally occurring anergic B cells?,” *Nat. Rev. Immunol.*, vol. 7, no. 8, pp. 633–643, Aug. 2007.

[39] G. K. Papadopoulos, C. Wijmenga, and F. Koning, “Interplay between genetics and the environment in the development of celiac disease: perspectives for a healthy life,” *J. Clin. Invest.*, vol. 108, no. 9, pp. 1261–1266, Nov. 2001.
[40] L. A. Zenewicz, C. Abraham, R. A. Flavell, and J. H. Cho, “Unraveling the Genetics of Autoimmunity,” *Cell*, vol. 140, no. 6, pp. 791–797, Mar. 2010.
[41] A. Marson, W. J. Housley, and D. A. Hafler, “Genetic basis of autoimmunity,” *Journal of Clinical Investigation*, vol. 125, no. 6, pp. 2234–2241, Jun. 2015.
[42] A. Goris and A. Liston, “The Immunogenetic Architecture of Autoimmune Disease,” *Cold Spring Harbor Perspectives in Biology*, vol. 4, no. 3, pp. a007260–a007260, Mar. 2012.
[43] K. Ghoreschi *et al.*, “Generation of pathogenic TH17 cells in the absence of TGF-β signalling,” *Nature*, vol. 467, no. 7318, pp. 967–971, Oct. 2010.
[44] K. Vandenbroeck, “Cytokine Gene Polymorphisms and Human Autoimmune Disease in the Era of Genome-Wide Association Studies,” *Journal of Interferon & Cytokine Research*, vol. 32, no. 4, pp. 139–151, Apr. 2012.
[45] M. Elliott *et al.*, “Ustekinumab: Lessons Learned from Targeting Interleukin-12/23p40 in Immune-Mediated Diseases,” *Annals of the New York Academy of Sciences*, vol. 1182, no. 1, pp. 97–110, Dec. 2009.
[46] K. A. Papp *et al.*, “Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 52-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 2),” *The Lancet*, vol. 371, no. 9625, pp. 1675–1684, May 2008.
[47] J. Aaltonen *et al.*, “An autoimmune disease, APECED, caused by mutations in a novel gene featuring two PHD-type zinc-finger domains,” *Nature Genetics*, vol. 17, no. 4, pp. 399–403, Dec. 1997.
[48] C. L. Bennett *et al.*, “The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3,” *Nature Genetics*, vol. 27, no. 1, pp. 20–21, Jan. 2001.
[49] M. S. Gresnigt and F. L. van de Veerdonk, “Biology of IL-36 cytokines and their role in disease,” *Seminars in Immunology*, vol. 25, no. 6, pp. 458–465, Dec. 2013.
[50] S. L. Taylor, B. R. Renshaw, K. E. Garka, D. E. Smith, and J. E. Sims, “Genomic Organization of the Interleukin-1 Locus,” *Genomics*, vol. 79, no. 5, pp. 726–733, May 2002.
[51] J. E. Towne *et al.*, “Interleukin-36 (IL-36) Ligands Require Processing for Full Agonist (IL-36α, IL-36β, and IL-36γ) or Antagonist (IL-36Ra) Activity,” *Journal of Biological Chemistry*, vol. 286, no. 49, pp. 42594–42602, Dec. 2011.
[52] J. S. Ainscough *et al.*, “Cathepsin S is the major activator of the psoriasis-associated proinflammatory cytokine IL-36γ,” *Proceedings of the National Academy of Sciences*, vol. 114, no. 13, pp. E2748–E2757, Mar. 2017.
[53] H. Wang *et al.*, “The activation and function of IL-36γ in neutrophilic inflammation in chronic rhinosinusitis,” *J. Allergy Clin. Immunol.*, vol. 141, no. 5, pp. 1646–1658, May 2018.
[54] T. Macleod *et al.*, “Neutrophil Elastase-mediated proteolysis activates the anti-inflammatory cytokine IL-36 Receptor antagonist,” *Scientific Reports*, vol. 6, no. 1, p. 24880, Jul. 2016.
[55] M. S. Gresnigt *et al.*, “The IL-36 receptor pathway regulates *Aspergillus fumigatus*- induced Th1 and Th17 responses: Immunity to infection,” *European Journal of Immunology*, vol. 43, no. 2, pp. 416–426, Feb. 2013.

[56] R. T. Chustz *et al.*, "Regulation and Function of the IL-1 Family Cytokine IL-1F9 in Human Bronchial Epithelial Cells," *American Journal of Respiratory Cell and Molecular Biology*, vol. 45, no. 1, pp. 145–153, Jul. 2011.

[57] J. B. Vos, M. A. van Sterkenburg, K. F. Rabe, J. Schalkwijk, P. S. Hiemstra, and N. A. Datson, "Transcriptional response of bronchial epithelial cells to *Pseudomonas aeruginosa* : identification of early mediators of host defense," *Physiological Genomics*, vol. 21, no. 3, pp. 324–336, May 2005.

[58] J. E. Sims and D. E. Smith, "The IL-1 family: regulators of immunity," *Nature Reviews Immunology*, vol. 10, no. 2, pp. 89–102, Feb. 2010.

[59] J. E. Towne, K. E. Garka, B. R. Renshaw, G. D. Virca, and J. E. Sims, "Interleukin (IL)-1F6, IL-1F8, and IL-1F9 Signal through IL-1Rrp2 and IL-1RAcP to Activate the Pathway Leading to NF-κB and MAPKs," *Journal of Biological Chemistry*, vol. 279, no. 14, pp. 13677–13688, Apr. 2004.

[60] J. E. Sims, S. Vigne, C. Gabay, and J. E. Towne, "IL-36: An Epithelial Cytokine Important in Psoriasis," in *Cytokine Frontiers*, T. Yoshimoto and T. Yoshimoto, Eds. Tokyo: Springer Japan, 2014, pp. 199–214.

[61] A. M. Foster *et al.*, "IL-36 promotes myeloid cell infiltration, activation, and inflammatory activity in skin," *J. Immunol.*, vol. 192, no. 12, pp. 6053–6061, Jun. 2014.

[62] A. Johnston *et al.*, "IL-1F5, -F6, -F8, and -F9: A Novel IL-1 Family Signaling System That Is Active in Psoriasis and Promotes Keratinocyte Antimicrobial Peptide Expression," *The Journal of Immunology*, vol. 186, no. 4, pp. 2613–2622, Feb. 2011.

[63] S. S. Saha *et al.*, "Signal Transduction and Intracellular Trafficking by the Interleukin 36 Receptor," *Journal of Biological Chemistry*, vol. 290, no. 39, pp. 23997–24006, Sep. 2015.

[64] A. Puel *et al.*, "Chronic Mucocutaneous Candidiasis in Humans with Inborn Errors of Interleukin-17 Immunity," *Science*, vol. 332, no. 6025, pp. 65–68, Apr. 2011.

[65] S. Vigne *et al.*, "IL-36 signaling amplifies Th1 responses by enhancing proliferation and Th1 polarization of naive CD4+ T cells," *Blood*, vol. 120, no. 17, pp. 3478–3487, Oct. 2012.

[66] N. Segueni *et al.*, "Limited Contribution of IL-36 versus IL-1 and TNF Pathways in Host Response to Mycobacterial Infection," *PLOS ONE*, vol. 10, no. 5, p. e0126058, May 2015.

[67] F. Ahsan, P. Moura-Alves, U. Guhlich-Bornhof, M. Klemm, S. H. E. Kaufmann, and J. Maertzdorf, "Role of Interleukin 36γ in Host Defense Against Tuberculosis," *Journal of Infectious Diseases*, vol. 214, no. 3, pp. 464–474, Aug. 2016.

[68] S. Vigne *et al.*, "IL-36R ligands are potent regulators of dendritic and T cells," *Blood*, vol. 118, no. 22, pp. 5813–5823, Nov. 2011.

[69] R. A. Ramadas, S. L. Ewart, Y. Iwakura, B. D. Medoff, and A. M. LeVine, "IL-36α Exerts Pro-Inflammatory Effects in the Lungs of Mice," *PLoS ONE*, vol. 7, no. 9, p. e45784, Sep. 2012.

[70] S. Mutamba, A. Allison, Y. Mahida, P. Barrow, and N. Foster, "Expression of IL-1Rrp2 by human myelomonocytic cells is unique to DCs and facilitates DC maturation by IL-1F8 and IL-1F9," *European Journal of Immunology*, vol. 42, no. 3, pp. 607–617, Mar. 2012.

[71] A. Harusato *et al.*, "IL-36γ signaling controls the induced regulatory T cell–Th9 cell balance via NFκB activation and STAT transcription factors," *Mucosal Immunology*, vol. 10, no. 6, pp. 1455–1467, Nov. 2017.

[72] H. Blumberg *et al.*, "Opposing activities of two novel members of the IL-1 ligand family regulate skin inflammation," *The Journal of Experimental Medicine*, vol. 204, no. 11, pp. 2603–2614, Oct. 2007.

[73] L. Tortola *et al.*, “Psoriasiform dermatitis is driven by IL-36–mediated DC-keratinocyte crosstalk,” *Journal of Clinical Investigation*, vol. 122, no. 11, pp. 3965–3976, Nov. 2012.
[74] N. Dand *et al.*, “Exome-wide association study reveals novel psoriasis susceptibility locus at TNFSF15 and rare protective alleles in genes contributing to type I IFN signalling,” *Human Molecular Genetics*, vol. 26, no. 21, pp. 4301–4313, Nov. 2017.
[75] P. Rahman *et al.*, “Association between the interleukin-1 family gene cluster and psoriatic arthritis,” *Arthritis Rheum.*, vol. 54, no. 7, pp. 2321–2325, Jul. 2006.
[76] S. Marrakchi *et al.*, “Interleukin-36–Receptor Antagonist Deficiency and Generalized Pustular Psoriasis,” *New England Journal of Medicine*, vol. 365, no. 7, pp. 620–628, Aug. 2011.
[77] A. Onoufriadis *et al.*, “Mutations in IL36RN/IL1F5 Are Associated with the Severe Episodic Inflammatory Skin Disease Known as Generalized Pustular Psoriasis,” *The American Journal of Human Genetics*, vol. 89, no. 3, pp. 432–437, Sep. 2011.
[78] K. Sugiura *et al.*, “The Majority of Generalized Pustular Psoriasis without Psoriasis Vulgaris Is Caused by Deficiency of Interleukin-36 Receptor Antagonist,” *Journal of Investigative Dermatology*, vol. 133, no. 11, pp. 2514–2521, Nov. 2013.
[79] K. Sugiura, “The genetic background of generalized pustular psoriasis: IL36RN mutations and CARD14 gain-of-function variants,” *Journal of Dermatological Science*, vol. 74, no. 3, pp. 187–192, Jun. 2014.
[80] N. Kanazawa, T. Nakamura, N. Mikita, and F. Furukawa, “Novel *IL36RN* mutation in a Japanese case of early onset generalized pustular psoriasis,” *The Journal of Dermatology*, vol. 40, no. 9, pp. 749–751, Sep. 2013.
[81] E. J. P. van Asseldonk *et al.*, “The Effect of the Interleukin-1 Cytokine Family Members IL-1F6 and IL-1F8 on Adipocyte Differentiation,” *Obesity*, vol. 18, no. 11, pp. 2234–2236, Nov. 2010.
[82] S. Frey *et al.*, “The novel cytokine interleukin-36α is expressed in psoriatic and rheumatoid arthritis synovium,” *Annals of the Rheumatic Diseases*, vol. 72, no. 9, pp. 1569–1574, Sep. 2013.
[83] S. Mai *et al.*, “Increased serum IL-36α and IL-36γ levels in patients with systemic lupus erythematosus: Association with disease activity and arthritis,” *International Immunopharmacology*, vol. 58, pp. 103–108, May 2018.
[84] S. E. Russell *et al.*, “IL-36α expression is elevated in ulcerative colitis and promotes colonic inflammation,” *Mucosal Immunology*, vol. 9, no. 5, pp. 1193–1204, Sep. 2016.
[85] K. Scheibe *et al.*, “Inhibiting Interleukin 36 Receptor Signaling Reduces Fibrosis in Mice With Chronic Intestinal Inflammation,” *Gastroenterology*, vol. 156, no. 4, pp. 1082-1097.e11, 2019.
[86] C. Gabay and J. E. Towne, “Regulation and function of interleukin-36 cytokines in homeostasis and pathological conditions,” *Journal of Leukocyte Biology*, vol. 97, no. 4, pp. 645–652, Apr. 2015.
[87] F. O. Nestle, D. H. Kaplan, and J. Barker, “Psoriasis,” *New England Journal of Medicine*, vol. 361, no. 5, pp. 496–509, Jul. 2009.
[88] E. Christophers, “Psoriasis - epidemiology and clinical spectrum,” *Clinical and Experimental Dermatology*, vol. 26, no. 4, pp. 314–320, Jun. 2001.
[89] R. Parisi, D. P. M. Symmons, C. E. M. Griffiths, and D. M. Ashcroft, “Global Epidemiology of Psoriasis: A Systematic Review of Incidence and Prevalence,” *Journal of Investigative Dermatology*, vol. 133, no. 2, pp. 377–385, Feb. 2013.
[90] S. K. Mahil, F. Capon, and J. N. Barker, “Genetics of Psoriasis,” *Dermatologic Clinics*, vol. 33, no. 1, pp. 1–11, Jan. 2015.

[91] A. S. Lønnberg, L. Skov, A. Skytthe, K. O. Kyvik, O. B. Pedersen, and S. F. Thomsen, "Heritability of psoriasis in a large twin sample," *British Journal of Dermatology*, vol. 169, no. 2, pp. 412–416, Aug. 2013.

[92] J. T. Elder, "The Genetics of Psoriasis," *Archives of Dermatology*, vol. 130, no. 2, p. 216, Feb. 1994.

[93] R. C. Trembath *et al.*, "Identification of a Major Susceptibility Locus on Chromosome 6p and Evidence for Further Disease Loci Revealed by a Two Stage Genome-Wide Search in Psoriasis," *Human Molecular Genetics*, vol. 6, no. 5, pp. 813–820, May 1997.

[94] C. D. Veal *et al.*, "Family-Based Analysis Using a Dense Single-Nucleotide Polymorphism–Based Map Defines Genetic Variation at PSORS1, the Major Psoriasis-Susceptibility Locus," *The American Journal of Human Genetics*, vol. 71, no. 3, pp. 554–564, Sep. 2002.

[95] R. Nair, "Evidence for two psoriasis susceptibility loci (HLA and 17q) and two novel candidate regions (16q and 20p) by genome-wide scan," *Human Molecular Genetics*, vol. 6, no. 8, pp. 1349–1356, Aug. 1997.

[96] B.-J. Feng *et al.*, "Multiple Loci within the Major Histocompatibility Complex Confer Risk of Psoriasis," *PLoS Genetics*, vol. 5, no. 8, p. e1000606, Aug. 2009.

[97] X. Yin *et al.*, "Genome-wide meta-analysis identifies multiple novel associations and ethnic heterogeneity of psoriasis susceptibility," *Nature Communications*, vol. 6, no. 1, Dec. 2015.

[98] Y. Okada *et al.*, "Fine Mapping Major Histocompatibility Complex Associations in Psoriasis and Its Clinical Subtypes," *The American Journal of Human Genetics*, vol. 95, no. 2, pp. 162–172, Aug. 2014.

[99] C. T. Jordan *et al.*, "PSORS2 Is Due to Mutations in CARD14," *The American Journal of Human Genetics*, vol. 90, no. 5, pp. 784–795, May 2012.

[100] Collaborative Association Study of Psoriasis (CASP) *et al.*, "Identification of 15 new psoriasis susceptibility loci highlights the role of innate immunity," *Nature Genetics*, vol. 44, no. 12, pp. 1341–1348, Dec. 2012.

[101] P. E. Stuart *et al.*, "Association of β-Defensin Copy Number and Psoriasis in Three Cohorts of European Origin," *Journal of Investigative Dermatology*, vol. 132, no. 10, pp. 2407–2413, Oct. 2012.

[102] R. de Cid *et al.*, "Deletion of the late cornified envelope LCE3B and LCE3C genes as a susceptibility factor for psoriasis," *Nature Genetics*, vol. 41, no. 2, pp. 211–215, Feb. 2009.

[103] F. Capon *et al.*, "Identification of ZNF313 / RNF114 as a novel psoriasis susceptibility gene," *Human Molecular Genetics*, vol. 17, no. 13, pp. 1938–1945, Jul. 2008.

[104] M. Cargill *et al.*, "A Large-Scale Genetic Association Study Confirms IL12B and Leads to the Identification of IL23R as Psoriasis-Risk Genes," *The American Journal of Human Genetics*, vol. 80, no. 2, pp. 273–290, Feb. 2007.

[105] L. C. Tsoi *et al.*, "Enhanced meta-analysis and replication studies identify five new psoriasis susceptibility loci," *Nature Communications*, vol. 6, no. 1, Dec. 2015.

[106] X. Zuo *et al.*, "Whole-exome SNP array identifies 15 new susceptibility loci for psoriasis," *Nature Communications*, vol. 6, no. 1, Dec. 2015.

[107] K. K.-H. Farh *et al.*, "Genetic and epigenetic fine mapping of causal autoimmune disease variants," *Nature*, vol. 518, no. 7539, pp. 337–343, Feb. 2015.

[108] J. A. Tennessen *et al.*, "Evolution and Functional Impact of Rare Coding Variation from Deep Sequencing of Human Exomes," *Science*, vol. 337, no. 6090, pp. 64–69, Jul. 2012.

[109] A. Coventry *et al.*, “Deep resequencing reveals excess rare recent variants consistent with explosive population growth,” *Nature Communications*, vol. 1, no. 8, p. 131, Nov. 2010.
[110] T. A. Manolio *et al.*, “Finding the missing heritability of complex diseases,” *Nature*, vol. 461, no. 7265, pp. 747–753, Oct. 2009.
[111] V. M. Narasimhan *et al.*, “Health and population effects of rare gene knockouts in adult humans with related parents,” *Science*, vol. 352, no. 6284, pp. 474–477, Apr. 2016.
[112] J. Funk, T. Langeland, E. Schrumpf, and L. E. Hanssen, “Psoriasis induced by interferon-α,” *British Journal of Dermatology*, vol. 125, no. 5, pp. 463–465, Nov. 1991.
[113] R. Lande *et al.*, “Plasmacytoid dendritic cells sense self-DNA coupled with antimicrobial peptide,” *Nature*, vol. 449, no. 7162, pp. 564–569, Oct. 2007.
[114] F. O. Nestle *et al.*, “Plasmacytoid predendritic cells initiate psoriasis through interferon-α production,” *The Journal of Experimental Medicine*, vol. 202, no. 1, pp. 135–143, Jul. 2005.
[115] L. C. Zaba *et al.*, “Psoriasis Is Characterized by Accumulation of Immunostimulatory and Th1/Th17 Cell-Polarizing Myeloid Dendritic Cells,” *Journal of Investigative Dermatology*, vol. 129, no. 1, pp. 79–88, Jan. 2009.
[116] H. Baurecht *et al.*, “Genome-wide Comparative Analysis of Atopic Dermatitis and Psoriasis Gives Insight into Opposing Genetic Mechanisms,” *The American Journal of Human Genetics*, vol. 96, no. 1, pp. 104–120, Jan. 2015.
[117] Y. Qian *et al.*, “The adaptor Act1 is required for interleukin 17–dependent signaling associated with autoimmune and inflammatory disease,” *Nature Immunology*, vol. 8, no. 3, pp. 247–256, Mar. 2007.
[118] R. Lande *et al.*, “The antimicrobial peptide LL37 is a T-cell autoantigen in psoriasis,” *Nature Communications*, vol. 5, no. 1, Dec. 2014.
[119] T. Mabuchi and N. Hirayama, “Binding Affinity and Interaction of LL-37 with HLA-C*06:02 in Psoriasis,” *Journal of Investigative Dermatology*, vol. 136, no. 9, pp. 1901–1903, Sep. 2016.
[120] A. Arakawa *et al.*, “Melanocyte antigen triggers autoimmunity in human psoriasis,” *The Journal of Experimental Medicine*, vol. 212, no. 13, pp. 2203–2212, Dec. 2015.
[121] M. Quaranta *et al.*, “Intraindividual genome expression analysis reveals a specific molecular signature of psoriasis and eczema,” *Science Translational Medicine*, vol. 6, no. 244, pp. 244ra90-244ra90, Jul. 2014.
[122] J. E. Greb *et al.*, “Psoriasis,” *Nature Reviews Disease Primers*, vol. 2, p. 16082, Nov. 2016.
[123] J. Bagel and L. S. Gold, “Combining Topical Psoriasis Treatment to Enhance Systemic and Phototherapy: A Review of the Literature,” *J Drugs Dermatol*, vol. 16, no. 12, pp. 1209–1222, 01 2017.
[124] D. Tartar, T. Bhutani, M. Huynh, T. Berger, and J. Koo, “Update on the immunological mechanism of action behind phototherapy,” *J Drugs Dermatol*, vol. 13, no. 5, pp. 564–568, May 2014.
[125] L. C. Zaba *et al.*, “Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses,” *The Journal of Experimental Medicine*, vol. 204, no. 13, pp. 3183–3194, Dec. 2007.
[126] L. C. Zaba *et al.*, “Effective treatment of psoriasis with etanercept is linked to suppression of IL-17 signaling, not immediate response TNF genes,” *Journal of Allergy and Clinical Immunology*, vol. 124, no. 5, pp. 1022-1030.e395, Nov. 2009.
[127] C. E. M. Griffiths *et al.*, “Comparison of Ustekinumab and Etanercept for Moderate-to-Severe Psoriasis,” *New England Journal of Medicine*, vol. 362, no. 2, pp. 118–128, Jan. 2010.

[128] C. L. Leonardi *et al.*, “Efficacy and safety of ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with psoriasis: 76-week results from a randomised, double-blind, placebo-controlled trial (PHOENIX 1),” *The Lancet*, vol. 371, no. 9625, pp. 1665–1674, May 2008.

[129] A. Blauvelt *et al.*, “Secukinumab is superior to ustekinumab in clearing skin of subjects with moderate-to-severe plaque psoriasis up to 1 year: Results from the CLEAR study,” *Journal of the American Academy of Dermatology*, vol. 76, no. 1, pp. 60-69.e9, Jan. 2017.

[130] R. G. Langley *et al.*, “Secukinumab in Plaque Psoriasis — Results of Two Phase 3 Trials,” *New England Journal of Medicine*, vol. 371, no. 4, pp. 326–338, Jul. 2014.

[131] C. E. M. Griffiths *et al.*, “Comparison of ixekizumab with etanercept or placebo in moderate-to-severe psoriasis (UNCOVER-2 and UNCOVER-3): results from two phase 3 randomised trials,” *The Lancet*, vol. 386, no. 9993, pp. 541–551, Aug. 2015.

[132] K. Papp *et al.*, “Tildrakizumab (MK-3222), an anti-interleukin-23p19 monoclonal antibody, improves psoriasis in a phase IIb randomized placebo-controlled trial,” *British Journal of Dermatology*, vol. 173, no. 4, pp. 930–939, Oct. 2015.

[133] H. Bachelez *et al.*, “Tofacitinib versus etanercept or placebo in moderate-to-severe chronic plaque psoriasis: a phase 3 randomised non-inferiority trial,” *The Lancet*, vol. 386, no. 9993, pp. 552–561, Aug. 2015.

[134] K. A. Papp *et al.*, “Tofacitinib, an oral Janus kinase inhibitor, for the treatment of chronic plaque psoriasis: Long-term efficacy and safety results from 2 randomized phase-III studies and 1 open-label long-term extension study,” *Journal of the American Academy of Dermatology*, vol. 74, no. 5, pp. 841–850, May 2016.

[135] S. K. Mahil, F. Capon, and J. N. Barker, “Update on psoriasis immunopathogenesis and targeted immunotherapy,” *Seminars in Immunopathology*, vol. 38, no. 1, pp. 11–27, Jan. 2016.

[136] A. Saggini, S. Chimenti, and A. Chiricozzi, “IL-6 as a Druggable Target in Psoriasis: Focus on Pustular Variants,” *Journal of Immunology Research*, vol. 2014, pp. 1–10, 2014.

[137] A. Robinson *et al.*, “Treatment of pustular psoriasis: From the Medical Board of the National Psoriasis Foundation,” *Journal of the American Academy of Dermatology*, vol. 67, no. 2, pp. 279–288, Aug. 2012.

[138] A. A. Navarini *et al.*, “European consensus statement on phenotypes of pustular psoriasis,” *J Eur Acad Dermatol Venereol*, vol. 31, no. 11, pp. 1792–1799, Nov. 2017.

[139] C. E. Griffiths and J. N. Barker, “Pathogenesis and clinical features of psoriasis,” *The Lancet*, vol. 370, no. 9583, pp. 263–271, Jul. 2007.

[140] S. Halevy, “Acute generalized exanthematous pustulosis:,” *Current Opinion in Allergy and Clinical Immunology*, vol. 9, no. 4, pp. 322–328, Aug. 2009.

[141] M. M. Speeckaert, R. Speeckaert, J. Lambert, and L. Brochez, “Acute generalized exanthematous pustulosis: an overview of the clinical, immunological and diagnostic concepts,” *European Journal of Dermatology*, no. 4, pp. 425–433, Jul. 2010.

[142] H. B. Naik and E. W. Cowen, “Autoinflammatory Pustular Neutrophilic Diseases,” *Dermatologic Clinics*, vol. 31, no. 3, pp. 405–425, Jul. 2013.

[143] S. Twelves *et al.*, “Clinical and genetic differences between pustular psoriasis subtypes,” *Journal of Allergy and Clinical Immunology*, vol. 143, no. 3, pp. 1021–1026, Mar. 2019.

[144] X. Li *et al.*, “Mutation analysis of the IL36RN gene in Chinese patients with generalized pustular psoriasis with/without psoriasis vulgaris,” *Journal of Dermatological Science*, vol. 76, no. 2, pp. 132–138, Nov. 2014.

[145] R. L. Giesey, G. R. Delost, T. R. Sharma, and K. D. Cooper, “Acute pustular eruption following a Jarisch-Herxheimer reaction in the treatment of syphilis,” *JAAD Case Reports*, vol. 4, no. 3, pp. 259–261, Apr. 2018.

[146] K. Asumalahti *et al.*, “Genetic Analysis of PSORS1 Distinguishes Guttate Psoriasis and Palmoplantar Pustulosis,” *Journal of Investigative Dermatology*, vol. 120, no. 4, pp. 627–632, Apr. 2003.

[147] S. Reddy *et al.*, “An Autoinflammatory Disease Due to Homozygous Deletion of the *IL1RN* Locus,” *New England Journal of Medicine*, vol. 360, no. 23, pp. 2438–2444, Jun. 2009.

[148] M. Farooq *et al.*, “Mutation Analysis of the *IL 36 RN* Gene in 14 Japanese Patients with Generalized Pustular Psoriasis,” *Human Mutation*, vol. 34, no. 1, pp. 176–183, Jan. 2013.

[149] D. M. Berki *et al.*, “Loss of IL36RN Function Does Not Confer Susceptibility to Psoriasis Vulgaris,” *Journal of Investigative Dermatology*, vol. 134, no. 1, pp. 271–273, Jan. 2014.

[150] J. L. Harden *et al.*, “CARD14 Expression in Dermal Endothelial Cells in Psoriasis,” *PLoS ONE*, vol. 9, no. 11, p. e111255, Nov. 2014.

[151] K. Sugiura, M. Muto, and M. Akiyama, “CARD14 c.526G>C (p.Asp176His) Is a Significant Risk Factor for Generalized Pustular Psoriasis with Psoriasis Vulgaris in the Japanese Cohort,” *Journal of Investigative Dermatology*, vol. 134, no. 6, pp. 1755–1757, Jun. 2014.

[152] P. Qin *et al.*, “Variant analysis of CARD14 in a Chinese Han population with psoriasis vulgaris and generalized pustular psoriasis,” *J. Invest. Dermatol.*, vol. 134, no. 12, pp. 2994–2996, Dec. 2014.

[153] S. K. Mahil *et al.*, “AP1S3 Mutations Cause Skin Autoinflammation by Disrupting Keratinocyte Autophagy and Up-Regulating IL-36 Production,” *J. Invest. Dermatol.*, vol. 136, no. 11, pp. 2251–2259, 2016.

[154] A. Diaz, “Deficiency of the Interleukin-36 Receptor Antagonist (DITRA) and Generalized Pustular Psoriasis,” in *Auto-Inflammatory Syndromes*, P. Efthimiou, Ed. Cham: Springer International Publishing, 2019, pp. 85–94.

[155] A. Johnston *et al.*, “IL-1 and IL-36 are dominant cytokines in generalized pustular psoriasis,” *Journal of Allergy and Clinical Immunology*, vol. 140, no. 1, pp. 109–120, Jul. 2017.

[156] C. M. Henry, G. P. Sullivan, D. M. Clancy, I. S. Afonina, D. Kulms, and S. J. Martin, “Neutrophil-Derived Proteases Escalate Inflammation through Activation of IL-36 Family Cytokines,” *Cell Reports*, vol. 14, no. 4, pp. 708–722, Feb. 2016.

[157] Y. Carrier *et al.*, “Inter-regulation of Th17 cytokines and the IL-36 cytokines in vitro and in vivo: implications in psoriasis pathogenesis,” *J. Invest. Dermatol.*, vol. 131, no. 12, pp. 2428–2437, Dec. 2011.

[158] C. Bridgewood, M. Stacey, A. Alase, D. Lagos, A. Graham, and M. Wittmann, “IL-36γ has proinflammatory effects on human endothelial cells,” *Exp. Dermatol.*, vol. 26, no. 5, pp. 402–408, 2017.

[159] M. Viguier, “Successful Treatment of Generalized Pustular Psoriasis With the Interleukin-1-Receptor Antagonist Anakinra: Lack of Correlation With IL1RN Mutations,” *Annals of Internal Medicine*, vol. 153, no. 1, p. 66, Jul. 2010.

[160] U. Hüffmeier, M. Wätzold, J. Mohr, M. P. Schön, and R. Mössner, “Successful therapy with anakinra in a patient with generalized pustular psoriasis carrying *IL36RN* mutations,” *British Journal of Dermatology*, vol. 170, no. 1, pp. 202–204, Jan. 2014.

[161] M. Tauber, M. Viguier, C. Le Gall, A. Smahi, and H. Bachelez, “Is it relevant to use an interleukin-1-inhibiting strategy for the treatment of patients with deficiency of interleukin-36

receptor antagonist?," *British Journal of Dermatology*, vol. 170, no. 5, pp. 1198–1199, May 2014.

[162] S. Imafuku *et al.*, "Efficacy and safety of secukinumab in patients with generalized pustular psoriasis: A 52-week analysis from phase III open-label multicenter Japanese study," *The Journal of Dermatology*, vol. 43, no. 9, pp. 1011–1017, Sep. 2016.

[163] K. Yamasaki, H. Nakagawa, Y. Kubo, K. Ootaki, and the Japanese Brodalumab Study Group, "Efficacy and safety of brodalumab in patients with generalized pustular psoriasis and psoriatic erythroderma: results from a 52-week, open-label study," *British Journal of Dermatology*, vol. 176, no. 3, pp. 741–751, Mar. 2017.

[164] H. Bachelez *et al.*, "Inhibition of the Interleukin-36 Pathway for the Treatment of Generalized Pustular Psoriasis," *New England Journal of Medicine*, vol. 380, no. 10, pp. 981–983, Mar. 2019.

[165] K. A. Zalocusky *et al.*, "The 10,000 Immunomes Project: Building a Resource for Human Immunology," *Cell Reports*, vol. 25, no. 2, pp. 513-522.e3, Oct. 2018.

[166] C. Q. F. Wang *et al.*, "IL-17 Induces Inflammation-Associated Gene Products in Blood Monocytes, and Treatment with Ixekizumab Reduces Their Expression in Psoriasis Patient Blood," *Journal of Investigative Dermatology*, vol. 134, no. 12, pp. 2990–2993, Dec. 2014.

[167] M. P. Rodero *et al.*, "Type I interferon-mediated autoinflammation due to DNase II deficiency," *Nature Communications*, vol. 8, no. 1, p. 2176, Dec. 2017.

[168] S. W. Canna *et al.*, "An activating NLRC4 inflammasome mutation causes autoinflammation with recurrent macrophage activation syndrome," *Nature Genetics*, vol. 46, no. 10, pp. 1140–1146, Oct. 2014.

[169] W. R. Swindell *et al.*, "Proteogenomic analysis of psoriasis reveals discordant and concordant changes in mRNA and protein abundance," *Genome Med*, vol. 7, no. 1, p. 86, 2015.

[170] D. Kim, G. Pertea, C. Trapnell, H. Pimentel, R. Kelley, and S. L. Salzberg, "TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions," *Genome Biology*, vol. 14, no. 4, p. R36, 2013.

[171] H. Li *et al.*, "The Sequence Alignment/Map format and SAMtools," *Bioinformatics*, vol. 25, no. 16, pp. 2078–2079, Aug. 2009.

[172] M. I. Love, W. Huber, and S. Anders, "Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2," *Genome Biology*, vol. 15, no. 12, p. 550, Dec. 2014.

[173] Y. Benjamini and Y. Hochberg, "On the Adaptive Control of the False Discovery Rate in Multiple Testing With Independent Statistics," *Journal of Educational and Behavioral Statistics*, vol. 25, no. 1, pp. 60–83, Mar. 2000.

[174] M. E. Ritchie *et al.*, "limma powers differential expression analyses for RNA-sequencing and microarray studies," *Nucleic Acids Research*, vol. 43, no. 7, pp. e47–e47, Apr. 2015.

[175] G. K. Smyth, "Linear Models and Empirical Bayes Methods for Assessing Differential Expression in Microarray Experiments," *Statistical Applications in Genetics and Molecular Biology*, vol. 3, no. 1, pp. 1–25, Jan. 2004.

[176] A. Krämer, J. Green, J. Pollard, and S. Tugendreich, "Causal analysis approaches in Ingenuity Pathway Analysis," *Bioinformatics*, vol. 30, no. 4, pp. 523–530, Feb. 2014.

[177] S. Li *et al.*, "Molecular signatures of antibody responses derived from a systems biology study of five human vaccines," *Nature Immunology*, vol. 15, no. 2, pp. 195–204, Feb. 2014.

[178] G. I. Rice *et al.*, "Assessment of Type I Interferon Signaling in Pediatric Inflammatory Disease," *Journal of Clinical Immunology*, vol. 37, no. 2, pp. 123–132, Feb. 2017.

[179] I. Rusinova *et al.*, "INTERFEROME v2.0: an updated database of annotated interferon-regulated genes," *Nucleic Acids Research*, vol. 41, no. D1, pp. D1040–D1046, Nov. 2012.
[180] S. K. Mahil *et al.*, "An analysis of IL-36 signature genes and individuals with *IL1RL2* knockout mutations validates IL-36 as a psoriasis therapeutic target," *Science Translational Medicine*, vol. 9, no. 411, p. eaan2514, Oct. 2017.
[181] A. Untergasser *et al.*, "Primer3—new capabilities and interfaces," *Nucleic Acids Research*, vol. 40, no. 15, pp. e115–e115, Aug. 2012.
[182] K. J. Livak and T. D. Schmittgen, "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method," *Methods*, vol. 25, no. 4, pp. 402–408, Dec. 2001.
[183] Y. Liang *et al.*, "Six-transmembrane epithelial antigens of the prostate comprise a novel inflammatory nexus in patients with pustular skin disorders," *Journal of Allergy and Clinical Immunology*, vol. 139, no. 4, pp. 1217–1227, Apr. 2017.
[184] M. Keermann, S. Kõks, E. Reimann, E. Prans, K. Abram, and K. Kingo, "Transcriptional landscape of psoriasis identifies the involvement of IL36 and IL36RN," *BMC Genomics*, vol. 16, p. 322, Apr. 2015.
[185] J. Kim *et al.*, "The Spectrum of Mild to Severe Psoriasis Vulgaris Is Defined by a Common Activation of IL-17 Pathway Genes, but with Key Differences in Immune Regulatory Genes," *Journal of Investigative Dermatology*, vol. 136, no. 11, pp. 2173–2182, Nov. 2016.
[186] C. M. Pfaff, Y. Marquardt, K. Fietkau, J. M. Baron, and B. Lüscher, "The psoriasis-associated IL-17A induces and cooperates with IL-36 cytokines to control keratinocyte differentiation and function," *Scientific Reports*, vol. 7, no. 1, p. 15631, Dec. 2017.
[187] W. Wang, X. Yu, C. Wu, and H. Jin, "IL-36γ inhibits differentiation and induces inflammation of keratinocyte via Wnt signaling pathway in psoriasis," *Int J Med Sci*, vol. 14, no. 10, pp. 1002–1007, 2017.
[188] B. Li *et al.*, "Transcriptome analysis of psoriasis in a large case-control sample: RNA-seq provides insights into disease mechanisms," *J. Invest. Dermatol.*, vol. 134, no. 7, pp. 1828–1838, Jul. 2014.
[189] A. Müller *et al.*, "IκBζ is a key transcriptional regulator of IL-36–driven psoriasis-related gene expression in keratinocytes," *Proceedings of the National Academy of Sciences*, vol. 115, no. 40, pp. 10088–10093, Oct. 2018.
[190] D.-D. Zhang *et al.*, "Long noncoding RNA LINC00305 promotes inflammation by activating the AHRR-NF-κB pathway in human monocytes," *Scientific Reports*, vol. 7, no. 1, p. 46204, Dec. 2017.
[191] E. K. Herter and N. Xu Landén, "Non-Coding RNAs: New Players in Skin Wound Healing," *Advances in Wound Care*, vol. 6, no. 3, pp. 93–107, Mar. 2017.
[192] M. K. Atianand *et al.*, "A Long Noncoding RNA lincRNA-EPS Acts as a Transcriptional Brake to Restrain Inflammation," *Cell*, vol. 165, no. 7, pp. 1672–1685, Jun. 2016.
[193] X. Mao, Z. Su, and A. K. Mookhtiar, "Long non-coding RNA: a versatile regulator of the nuclear factor- *κ* B signalling circuit," *Immunology*, vol. 150, no. 4, pp. 379–388, Apr. 2017.
[194] S. Carpenter *et al.*, "A Long Noncoding RNA Mediates Both Activation and Repression of Immune Response Genes," *Science*, vol. 341, no. 6147, pp. 789–792, Aug. 2013.
[195] L. C. Tsoi *et al.*, "Analysis of long non-coding RNAs highlights tissue-specific expression patterns and epigenetic profiles in normal and psoriatic skin," *Genome Biology*, vol. 16, no. 1, p. 24, 2015.

[196] R. Speeckaert, J. Lambert, L. Grine, M. Van Gele, S. De Schepper, and N. van Geel, “The many faces of interleukin-17 in inflammatory skin diseases,” *British Journal of Dermatology*, vol. 175, no. 5, pp. 892–901, Nov. 2016.
[197] J. Kim and J. G. Krueger, “Highly Effective New Treatments for Psoriasis Target the IL-23/Type 17 T Cell Autoimmune Axis,” *Annual Review of Medicine*, vol. 68, no. 1, pp. 255–269, Jan. 2017.
[198] M. A. Lowes, C. B. Russell, D. A. Martin, J. E. Towne, and J. G. Krueger, “The IL-23/T17 pathogenic axis in psoriasis is amplified by keratinocyte responses,” *Trends in Immunology*, vol. 34, no. 4, pp. 174–181, Apr. 2013.
[199] T. Sano *et al.*, “An IL-23R/IL-22 Circuit Regulates Epithelial Serum Amyloid A to Promote Local Effector Th17 Responses,” *Cell*, vol. 163, no. 2, pp. 381–393, Oct. 2015.
[200] K. Ohko, K. Nakajima, S. Kataoka, M. Takaishi, and S. Sano, “IL-36 Signaling Is Essential for Psoriatic Inflammation through the Augmentation of Innate Immune Responses,” *Journal of Investigative Dermatology*, p. S0022202X18329427, Dec. 2018.
[201] Z. Su *et al.*, “IL-36 receptor antagonistic antibodies inhibit inflammatory responses in preclinical models of psoriasiform dermatitis,” *Experimental Dermatology*, vol. 28, no. 2, pp. 113–120, Feb. 2019.
[202] R. Ganesan *et al.*, “Generation and functional characterization of anti-human and anti-mouse IL-36R antagonist monoclonal antibodies,” *mAbs*, vol. 9, no. 7, pp. 1143–1154, Oct. 2017.
[203] C. T. Ng, J. L. Mendoza, K. C. Garcia, and M. B. A. Oldstone, “Alpha and Beta Type 1 Interferon Signaling: Passage for Diverse Biologic Outcomes,” *Cell*, vol. 164, no. 3, pp. 349–352, Jan. 2016.
[204] K.-K. Conzelmann, “Transcriptional Activation of Alpha/Beta Interferon Genes: Interference by Nonsegmented Negative-Strand RNA Viruses,” *Journal of Virology*, vol. 79, no. 9, pp. 5241–5248, May 2005.
[205] K. Honda *et al.*, “IRF-7 is the master regulator of type-I interferon-dependent immune responses,” *Nature*, vol. 434, no. 7034, pp. 772–777, Apr. 2005.
[206] Y. J. Crow and N. Manel, “Aicardi–Goutières syndrome and the type I interferonopathies,” *Nature Reviews Immunology*, vol. 15, no. 7, pp. 429–440, Jul. 2015.
[207] J. Hua, K. Kirou, C. Lee, and M. K. Crow, “Functional assay of type I interferon in systemic lupus erythematosus plasma and association with anti–RNA binding protein autoantibodies,” *Arthritis & Rheumatism*, vol. 54, no. 6, pp. 1906–1916, Jun. 2006.
[208] Y.-J. Seo *et al.*, “Validation of a HeLa *Mx2*/Luc Reporter Cell Line for the Quantification of Human Type I Interferons,” *Pharmacology*, vol. 84, no. 3, pp. 135–144, 2009.
[209] R. S. Spielman, L. A. Bastone, J. T. Burdick, M. Morley, W. J. Ewens, and V. G. Cheung, “Common genetic variants account for differences in gene expression among ethnic groups,” *Nature Genetics*, vol. 39, no. 2, pp. 226–231, Feb. 2007.
[210] P. Alvarez and L. E. Jensen, “Imiquimod Treatment Causes Systemic Disease in Mice Resembling Generalized Pustular Psoriasis in an IL-1 and IL-36 Dependent Manner,” *Mediators of Inflammation*, vol. 2016, pp. 1–10, 2016.
[211] S. Hussain *et al.*, “IL36RN mutations define a severe autoinflammatory phenotype of generalized pustular psoriasis,” *Journal of Allergy and Clinical Immunology*, vol. 135, no. 4, pp. 1067-1070.e9, Apr. 2015.
[212] K. D. Mayer-Barber and B. Yan, “Clash of the Cytokine Titans: counter-regulation of interleukin-1 and type I interferon-mediated inflammatory responses,” *Cellular & Molecular Immunology*, vol. 14, no. 1, pp. 22–35, Jan. 2017.

[213] A. Arakawa *et al.*, “Unopposed IL-36 Activity Promotes Clonal CD4+ T-Cell Responses with IL-17A Production in Generalized Pustular Psoriasis,” *Journal of Investigative Dermatology*, vol. 138, no. 6, pp. 1338–1347, Jun. 2018.

[214] S. Ikeda *et al.*, “Therapeutic depletion of myeloid lineage leukocytes in patients with generalized pustular psoriasis indicates a major role for neutrophils in the immunopathogenesis of psoriasis,” *Journal of the American Academy of Dermatology*, vol. 68, no. 4, pp. 609–617, Apr. 2013.

[215] M. Chu *et al.*, “Elevated Expression and Pro-Inflammatory Activity of IL-36 in Patients with Systemic Lupus Erythematosus,” *Molecules*, vol. 20, no. 10, pp. 19588–19604, Oct. 2015.

[216] E. Tonti *et al.*, “Follicular Helper NKT Cells Induce Limited B Cell Responses and Germinal Center Formation in the Absence of CD4+ T Cell Help,” *The Journal of Immunology*, vol. 188, no. 7, pp. 3217–3222, Apr. 2012.

[217] A. Cerutti, M. Cols, and I. Puga, “Marginal zone B cells: virtues of innate-like antibody-producing lymphocytes,” *Nature Reviews Immunology*, vol. 13, no. 2, pp. 118–132, Feb. 2013.

[218] E. Y. Bassoy, J. E. Towne, and C. Gabay, “Regulation and function of interleukin-36 cytokines,” *Immunological Reviews*, vol. 281, no. 1, pp. 169–178, Jan. 2018.

[219] A. H. Talukder *et al.*, “Phospholipid Scramblase 1 regulates Toll-like receptor 9-mediated type I interferon production in plasmacytoid dendritic cells,” *Cell Research*, vol. 22, no. 7, pp. 1129–1139, Jul. 2012.

[220] J. Li *et al.*, “Interferon-α priming promotes lipid uptake and macrophage-derived foam cell formation: A novel link between interferon-α and atherosclerosis in lupus,” *Arthritis & Rheumatism*, vol. 63, no. 2, pp. 492–502, Feb. 2011.

[221] C. Bridgewood *et al.*, “IL-36γ Is a Strong Inducer of IL-23 in Psoriatic Cells and Activates Angiogenesis,” *Frontiers in Immunology*, vol. 9, p. 200, Feb. 2018.

[222] T. Elango, A. Thirupathi, S. Subramanian, P. Ethiraj, H. Dayalan, and P. Gnanaraj, “Methotrexate treatment provokes apoptosis of proliferating keratinocyte in psoriasis patients,” *Clin. Exp. Med.*, vol. 17, no. 3, pp. 371–381, Aug. 2017.

[223] G. K. Perera, P. Di Meglio, and F. O. Nestle, “Psoriasis,” *Annual Review of Pathology: Mechanisms of Disease*, vol. 7, no. 1, pp. 385–422, Feb. 2012.

[224] M. Mele *et al.*, “The human transcriptome across tissues and individuals,” *Science*, vol. 348, no. 6235, pp. 660–665, May 2015.

[225] D. W. Huang *et al.*, “DAVID Bioinformatics Resources: expanded annotation database and novel algorithms to better extract biology from large gene lists,” *Nucleic Acids Research*, vol. 35, no. suppl_2, pp. W169–W175, Jul. 2007.

[226] A. Subramanian *et al.*, “Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles,” *Proceedings of the National Academy of Sciences*, vol. 102, no. 43, pp. 15545–15550, Oct. 2005.

[227] J. M. Stuart, “A Gene-Coexpression Network for Global Discovery of Conserved Genetic Modules,” *Science*, vol. 302, no. 5643, pp. 249–255, Oct. 2003.

[228] M. Franz *et al.*, “GeneMANIA update 2018,” *Nucleic Acids Research*, vol. 46, no. W1, pp. W60–W64, Jul. 2018.

[229] B. B. Cummings *et al.*, “Improving genetic diagnosis in Mendelian disease with transcriptome sequencing,” *Science Translational Medicine*, vol. 9, no. 386, p. eaal5209, Apr. 2017.

[230] L. S. Kremer *et al.*, “Genetic diagnosis of Mendelian disorders via RNA sequencing,” *Nature Communications*, vol. 8, p. 15824, Jun. 2017.

[231] A. K. Shalek *et al.*, “Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells,” *Nature*, vol. 498, no. 7453, pp. 236–240, Jun. 2013.
[232] LifeLines Cohort Study *et al.*, “Single-cell RNA sequencing identifies celltype-specific cis-eQTLs and co-expression QTLs,” *Nature Genetics*, vol. 50, no. 4, pp. 493–497, Apr. 2018.
[233] B. Germán *et al.*, “Disrupting the IL-36 and IL-23/IL-17 loop underlies the efficacy of calcipotriol and corticosteroid therapy for psoriasis,” *JCI Insight*, vol. 4, no. 2, p. e123390, Jan. 2019.
[234] L. Mercurio *et al.*, “IL-38 has an anti-inflammatory action in psoriasis and its expression correlates with disease severity and therapeutic response to anti-IL-17A treatment,” *Cell Death & Disease*, vol. 9, no. 11, p. 1104, Nov. 2018.
[235] B. Meier-Schiesser *et al.*, “Culprit Drugs Induce Specific IL-36 Overexpression in Acute Generalized Exanthematous Pustulosis,” *Journal of Investigative Dermatology*, vol. 139, no. 4, pp. 848–858, Apr. 2019.
[236] X. Zhang, “Regulatory functions of innate-like B cells,” *Cellular & Molecular Immunology*, vol. 10, no. 2, pp. 113–121, Mar. 2013.

9 Appendix

Appendix A: Enriched Pathways in KCs stimulated with IL-36

Ingenuity Canonical Pathway	FDR_IL-36α	FDR_IL-36β	FDR_IL-36γ	Enriched in all three IL-36 datasets
Acute Phase Response Signaling	3.07E-06	1.52E-05	1.87E-07	YES
Agranulocyte Adhesion and Diapedesis	1.76E-07	6.93E-07	1.17E-07	YES
Airway Pathology in Chronic Obstructive Pulmonary Disease	4.21E-04	2.11E-03	5.12E-04	YES
Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	3.01E-05	1.00E-03	5.12E-05	YES
Antioxidant Action of Vitamin C	3.06E-03	2.35E-03	8.32E-04	YES
Atherosclerosis Signaling	1.70E-10	2.46E-10	5.76E-11	YES
Bladder Cancer Signaling	8.52E-03	NA	1.09E-02	NO
CCR3 Signaling in Eosinophils	NA	2.53E-02	4.81E-02	NO
Communication between Innate and Adaptive Immune Cells	1.63E-03	1.66E-02	3.93E-04	YES
Complement System	2.41E-02	2.60E-03	3.54E-03	YES
Dendritic Cell Maturation	7.86E-04	6.81E-03	3.39E-04	YES
Differential Regulation of Cytokine Production in Intestinal Epithelial Cells by IL-17A and IL-17F	3.03E-05	4.00E-04	4.58E-05	YES
Differential Regulation of Cytokine Production in Macrophages and T Helper Cells by IL-17A and IL-17F	4.78E-02	NA	NA	NO
Eicosanoid Signaling	3.01E-03	2.18E-04	6.23E-04	YES
Estrogen Biosynthesis	2.71E-02	NA	3.14E-02	NO
Fatty Acid Î±-oxidation	NA	2.53E-02	NA	NO
Fc Epsilon RI Signaling	NA	1.66E-02	8.35E-03	NO
FXR/RXR Activation	1.63E-03	6.70E-03	8.71E-05	YES
Glycogen Degradation III	2.80E-02	NA	3.14E-02	NO
Graft-versus-Host Disease Signaling	6.80E-03	3.44E-02	8.33E-03	YES
Granulocyte Adhesion and Diapedesis	1.50E-08	4.74E-08	6.09E-10	YES
Hematopoiesis from Pluripotent Stem Cells	4.04E-02	NA	7.76E-03	NO

Hepatic Cholestasis	8.93E-06	2.35E-03	1.98E-05	YES
Hepatic Fibrosis / Hepatic Stellate Cell Activation	2.61E-05	5.42E-04	2.55E-04	YES
HMGB1 Signaling	3.82E-02	NA	NA	NO
IL-10 Signaling	4.24E-04	5.81E-03	6.61E-04	YES
IL-17A Signaling in Airway Cells	9.77E-05	4.44E-04	2.41E-05	YES
IL-17A Signaling in Fibroblasts	1.87E-04	2.93E-04	2.41E-05	YES
IL-17A Signaling in Gastric Cells	9.33E-03	3.44E-02	8.86E-04	YES
IL-17 Signaling	3.80E-02	NA	1.88E-03	NO
IL-1 Signaling	9.98E-03	NA	1.26E-02	NO
IL-6 Signaling	4.59E-05	2.11E-03	8.94E-05	YES
IL-8 Signaling	1.51E-02	8.33E-03	6.74E-03	YES
Inflammasome pathway	2.38E-05	2.93E-04	3.07E-05	YES
iNOS Signaling	5.06E-03	5.10E-03	6.43E-03	YES
Leukocyte Extravasation Signaling	NA	1.23E-02	NA	NO
LPS/IL-1 Mediated Inhibition of RXR Function	NA	6.59E-03	3.71E-02	NO
LXR/RXR Activation	6.35E-06	9.15E-05	1.74E-06	YES
Lymphotoxin Î² Receptor Signaling	1.90E-02	NA	2.24E-02	NO
MIF-mediated Glucocorticoid Regulation	1.24E-05	1.92E-06	1.34E-06	YES
MIF Regulation of Innate Immunity	3.14E-05	8.46E-06	5.02E-06	YES
NF-ÎºB Signaling	2.38E-05	1.74E-03	4.58E-05	YES
p38 MAPK Signaling	5.75E-06	1.09E-05	1.34E-06	YES
Phospholipase C Signaling	NA	9.77E-03	1.67E-02	NO
Phospholipases	1.51E-02	3.85E-03	2.94E-03	YES
PPAR Signaling	1.98E-03	2.00E-02	2.82E-03	YES
RAR Activation	1.32E-02	NA	5.60E-03	NO
Retinoate Biosynthesis I	2.06E-03	1.20E-02	2.70E-03	YES
Retinol Biosynthesis	2.80E-02	NA	NA	NO
Role of Cytokines in Mediating Communication between Immune Cells	1.06E-07	7.32E-06	2.07E-07	YES
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	4.69E-04	4.80E-03	6.45E-05	YES

Role of IL-17A in Arthritis	6.35E-06	2.18E-04	1.02E-05	YES
Role of IL-17A in Psoriasis	8.56E-14	9.83E-12	2.30E-13	YES
Role of IL-17F in Allergic Inflammatory Airway Diseases	4.50E-05	8.54E-04	7.63E-06	YES
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	1.02E-04	1.70E-03	2.02E-05	YES
Role of MAPK Signaling in the Pathogenesis of Influenza	2.35E-02	6.81E-03	8.32E-04	YES
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	3.08E-02	NA	1.52E-02	NO
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	2.46E-03	3.16E-03	8.32E-04	YES
Role of Tissue Factor in Cancer	NA	NA	9.21E-03	NO
Sperm Motility	NA	6.59E-03	2.41E-03	NO
Synaptic Long Term Depression	NA	4.27E-02	1.98E-02	NO
TNFR1 Signaling	7.10E-03	3.62E-02	8.57E-03	YES
TNFR2 Signaling	1.26E-03	7.11E-03	1.57E-03	YES
Toll-like Receptor Signaling	1.06E-07	8.70E-06	2.07E-07	YES
TREM1 Signaling	8.52E-05	4.00E-04	1.46E-04	YES
TWEAK Signaling	2.00E-02	NA	2.23E-02	NO
VDR/RXR Activation	NA	NA	7.57E-03	NO
VEGF Family Ligand-Receptor Interactions	4.04E-02	1.66E-02	1.12E-02	YES
Xenobiotic Metabolism Signaling	NA	3.34E-02	NA	NO

NA refers to pathways generating an FDR>0.05

Appendix B: Genes significantly up-regulated in GPP whole blood

Symbol	log2FoldChange	P value	FDR
CD177	3.04E+00	5.28E-06	8.40E-03
MMP9	2.40E+00	2.67E-04	3.11E-02
IFITM3	1.96E+00	1.72E-04	2.68E-02
FCGR1A	1.95E+00	3.25E-04	3.39E-02
TNFAIP6	1.84E+00	5.08E-06	8.40E-03
LINC01506	1.67E+00	5.51E-05	2.22E-02
FCGR1B	1.65E+00	7.17E-05	2.42E-02
IL1R2	1.63E+00	1.12E-04	2.42E-02
RAB20	1.59E+00	8.79E-05	2.42E-02
LINC00694	1.58E+00	2.23E-04	2.81E-02
SERPING1	1.56E+00	4.11E-08	3.26E-04
BTNL8	1.53E+00	5.12E-06	8.40E-03
VNN2	1.47E+00	1.85E-04	2.68E-02
PLSCR1	1.45E+00	1.29E-05	1.02E-02
LY96	1.44E+00	1.67E-04	2.68E-02
GRINA	1.40E+00	1.79E-04	2.68E-02
CEBPD	1.39E+00	2.06E-05	1.17E-02
DSC2	1.36E+00	5.06E-04	4.23E-02
FCAR	1.35E+00	4.05E-04	3.67E-02
NAMPT	1.34E+00	2.88E-04	3.14E-02
DUSP1	1.32E+00	1.54E-05	1.02E-02
TREM1	1.31E+00	9.28E-05	2.42E-02
FUT7	1.31E+00	4.07E-04	3.67E-02
KREMEN1	1.29E+00	1.53E-05	1.02E-02
PLAUR	1.29E+00	1.14E-04	2.42E-02
SCO2	1.28E+00	8.80E-06	9.99E-03
SAT1	1.28E+00	8.23E-05	2.42E-02
IFI6	1.27E+00	5.57E-04	4.45E-02
OASL	1.26E+00	1.16E-05	1.02E-02
SECTM1	1.25E+00	1.67E-04	2.68E-02
SH2B2	1.25E+00	1.22E-04	2.42E-02
IL1R1	1.24E+00	6.62E-04	4.79E-02
MIR223	1.23E+00	1.08E-04	2.42E-02
BCL3	1.21E+00	3.97E-04	3.67E-02
CEBPB	1.21E+00	1.19E-04	2.42E-02
TGFA	1.21E+00	5.02E-05	2.22E-02
BRI3	1.21E+00	2.48E-04	2.98E-02
NINJ1	1.21E+00	1.73E-04	2.68E-02
IFIT3	1.20E+00	3.53E-04	3.47E-02
TXN	1.19E+00	1.13E-04	2.42E-02
NFIL3	1.18E+00	6.06E-04	4.50E-02
FPR2	1.18E+00	7.43E-04	4.98E-02

CCDC71L	1.16E+00	4.82E-04	4.07E-02
PILRA	1.15E+00	8.89E-05	2.42E-02
TMEM120A	1.15E+00	9.97E-05	2.42E-02
LST1	1.15E+00	2.22E-04	2.81E-02
CD14	1.14E+00	2.70E-04	3.11E-02
CSRNP1	1.14E+00	1.96E-04	2.68E-02
SCARF1	1.12E+00	5.65E-04	4.45E-02
KLHDC8B	1.11E+00	1.89E-04	2.68E-02
CYP27A1	1.11E+00	7.73E-06	9.99E-03
SMARCD3	1.10E+00	4.64E-04	4.01E-02
NCF1	1.09E+00	3.21E-04	3.39E-02
ASPRV1	1.09E+00	2.83E-04	3.12E-02
CFP	1.08E+00	9.23E-05	2.42E-02
ZNF467	1.08E+00	2.80E-04	3.12E-02
STX11	1.08E+00	3.23E-05	1.60E-02
TMEM140	1.07E+00	1.17E-04	2.42E-02
TSC22D3	1.07E+00	2.54E-05	1.34E-02
VNN3	1.06E+00	6.72E-04	4.81E-02
CCR1	1.05E+00	3.46E-04	3.44E-02
SLC16A3	1.04E+00	4.13E-04	3.69E-02
CEP19	1.04E+00	1.66E-04	2.68E-02
GLIPR2	1.04E+00	6.03E-04	4.50E-02
DDX60L	9.71E-01	1.68E-04	2.68E-02
LAT2	9.70E-01	4.35E-04	3.83E-02
F2RL1	9.60E-01	9.49E-05	2.42E-02
MXD1	9.49E-01	6.31E-04	4.60E-02
HIP1	9.41E-01	8.77E-05	2.42E-02
WAS	9.39E-01	6.02E-04	4.50E-02
B4GALT5	9.39E-01	4.74E-04	4.05E-02
IL10RB	9.30E-01	6.84E-04	4.85E-02
NFAM1	9.30E-01	5.18E-04	4.25E-02
PARP9	9.22E-01	1.52E-05	1.02E-02
TMEM164	9.16E-01	2.77E-04	3.12E-02
IFIT2	9.12E-01	3.28E-04	3.39E-02
FAM214B	9.00E-01	5.94E-04	4.50E-02
RNF130	8.99E-01	2.69E-04	3.11E-02
CTSL	8.96E-01	2.01E-04	2.71E-02
HSD17B11	8.96E-01	1.80E-04	2.68E-02
SRA1	8.91E-01	5.78E-04	4.49E-02
SHISA5	8.90E-01	6.52E-05	2.42E-02
IL13RA1	8.71E-01	3.39E-04	3.41E-02
CTSB	8.64E-01	3.74E-04	3.58E-02
CHMP5	8.63E-01	8.97E-05	2.42E-02
PSMB9	8.55E-01	6.80E-05	2.42E-02

STAB1	8.55E-01	2.29E-04	2.85E-02
IRF1	8.46E-01	1.87E-05	1.14E-02
RHBDD2	8.34E-01	1.87E-04	2.68E-02
ODF3B	8.26E-01	6.91E-04	4.86E-02
ERGIC1	8.19E-01	5.43E-04	4.41E-02
DGAT1	7.87E-01	1.58E-04	2.68E-02
MX2	7.86E-01	3.39E-04	3.41E-02
SOCS3	7.79E-01	3.79E-04	3.58E-02
SOWAHD	7.76E-01	2.05E-04	2.71E-02
EEPD1	7.69E-01	1.95E-04	2.68E-02
NMI	7.47E-01	4.38E-04	3.83E-02
NOD2	7.44E-01	5.12E-05	2.22E-02
NEU1	7.44E-01	7.10E-04	4.91E-02
MYADM	7.33E-01	2.42E-04	2.95E-02
TAP1	7.29E-01	1.86E-04	2.68E-02
GBP5	7.07E-01	5.82E-04	4.49E-02
GALNT3	6.83E-01	5.19E-04	4.25E-02
PSMB8	6.75E-01	1.39E-04	2.63E-02
APOL2	6.68E-01	2.95E-04	3.17E-02
APOL1	6.67E-01	5.60E-05	2.22E-02
STK19	6.46E-01	1.14E-04	2.42E-02
CMTM7	6.39E-01	1.27E-04	2.46E-02
H2AFY	6.07E-01	7.34E-04	4.98E-02
SEC61B	5.67E-01	7.46E-04	4.98E-02
SF3B5	4.07E-01	2.15E-04	2.80E-02
TMPO	-4.27E-01	5.59E-04	4.45E-02
CARNS1	-6.35E-01	3.59E-04	3.48E-02
ZNF37BP	-6.65E-01	7.02E-04	4.90E-02
PLEKHA1	-7.41E-01	7.41E-04	4.98E-02
CLUHP3	-9.01E-01	6.13E-04	4.51E-02
NELL2	-1.02E+00	1.78E-04	2.68E-02

Appendix C: Publications arising from this work

An analysis of IL-36 signature genes and individuals with IL1RL2 knockout mutations validates IL-36 as a psoriasis therapeutic target

Satveer K. Mahil, **Marika Catapano**, Paola Di Meglio, Nick Dand, Helena Ahlfors, Ian M. Carr, Catherine H. Smith, Richard C. Trembath, Mark Peakman, John Wright, Francesca D. Ciccarelli, Jonathan N. Barker, Francesca Capon

Published 11 October 2017, Sci. Transl. Med. 9, eaan2514 (2017)

DOI: 10.1126/scitranslmed.aan2514